THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 106 NOVEMBER 1, 1933 No. 2

REFLEX ACTIVATION OF THE SYMPATHETIC SYSTEM IN THE SPINAL CAT

CHANDLER M. BROOKS[1]

From the Laboratories of Physiology in the Harvard Medical School

Received for publication July 3, 1933

Activity of the sympathetic nervous system can be produced in a normal animal by nociceptive stimuli, asphyxiation, exposure to cold, hemorrhage, insulin hypoglycemia, and emotional excitement (Cannon, 1929). The sympathetic activity resulting from any one of these stimuli gives rise to an increased arterial pressure and blood-sugar concentration, cardiac acceleration, reduced blood-clotting time, pupilary dilatation, retraction of the denervated nicitating membrane, etc. It is often possible to obtain a quantitative estimation of the intensity of the sympathetic activity and the resulting adrenal discharge by measuring these changes (Elliott, 1912; Cannon and Rapport, 1921; Rosenblueth, 1932).

Gaskell (1886) and later Langley (1900) demonstrated that the sympathetic outflow from the central nervous system is confined to preganglionic fibers having their origin in the thoracic and upper lumbar regions of the cord. There is available ample evidence for the existence of hypothalamic and bulbar mechanisms which are involved in the excitation and control of the activity of this peripheral part of the sympathetic system (Bard, 1929). That diencephalic centers exist which are capable of activating the sympathetic system is evident from the work of Bard (1928) and Beattie, Brow and Long (1930). Keller (1932) has found that in chronic midbrain animals signs of sympathetic activity were readily induced by physiological stimuli. This observation indicates that the medulla contains the essential centers for some sympathetic responses. The work of Dittmar (1873), Owsjannikow (1871), and Ranson and Billingsley (1916) has shown that there is a bulbar mechanism which makes a definite connection with the sympathetic outflow and is essential to the maintenance of the normal vasoconstrictor tone and to normal reflex

[1] Medical Fellow of the National Research Council.

changes in blood pressure. Elliott (1912), Cannon and Rapport (1921), and Tournade and Malméjac (1932) have demonstrated that the medulla contains a center responsible for the reflex secretion of adrenin and that this center is subject to reflex inhibition as well as excitation.

Griffith (1923) ascertained that the rise in blood sugar due to stimulation of an afferent nerve was largely the result of reflex excitation of the sympathico-adrenal system. An effort was made by Brooks (1931) to delimit the central nervous mechanism essential to this reflex hyperglycemia. It was found that removal of the cerebral hemispheres and portions of the brain stem rostral to the brachium pontis did not diminish the response, but that a transection just caudal to the brachium pontis practically abolished the reflex. This furnished additional evidence of a bulbar center which is normally involved in reflex excitation of the sympathetic nervous system.

Transection of the medulla posterior to the pons, however, did not completely abolish the reflex changes in blood sugar. Likewise, in a small percentage of the decapitate animals studied a slight rise in blood sugar accompanied by a clear increase in heart rate and a rise in blood pressure was obtained upon central stimulation of the brachial or crural nerve. It was thought that spinal shock and the disturbances due to the operative procedure, rather than the absence of a bulbar reflex center, might explain the failure of the nociceptive stimuli to excite the sympathetic system to discharge with normal intensity.

It has long been known that the arterial pressure of a spinal animal can be further lowered by pithing the cord or cutting the splanchnics (Tigerstedt, 1923). Sherrington (1906) observed that following a cervical section of the cord vascular tone and pressor reflexes, which at first were lost, reappeared after a short time. The work of Ranson (1916), which furnished evidence of a primary bulbar vasoconstrictor center, also revealed the presence of a secondary spinal vasoconstrictor mechanism. This made it not unreasonable to seek for further evidence of a functional spinal representation of the sympathetic nervous system. Therefore a qualitative and quantitative study was made of the sympathetic activity that could be elicited in animals in which the spinal cord had been transected at the level of the sixth cervical vertebra three days to two weeks previously. Various stimuli were used that would have produced strong sympathetic activity from animals in which the higher centers of the brain retained their connection with the thoraco-lumbar sympathetic outflow. This paper is concerned with the results obtained on stimulation of the sciatic nerve centrally.

Method. Transections of the spinal cord were performed aseptically under pentobarbital sodium (0.7 cc. per kgm.) anesthesia. Animals in which a cervical transection had been made were very difficult to keep in

good condition for more than two weeks because of their inability to regulate their body temperature and because of nutrition and elimination difficulties. It was found that after the third or fourth day little change could be noticed in the reflex activities of the cord, in the height of blood pressure or in the results obtained from the experimental procedure to be described later. Consequently, in the majority of cases, the effect of afferent stimulation was tested on the third day after the cord had been transected. Some animals, however, were not used until the end of the second week. After low thoracic and lumbar transections the animals were not completely helpless and they were all kept for over a week before being experimented on.

Female cats were used when possible because of the ease with which they could be catheterized. Each day the bladder had to be emptied by catheterization or pressure, though defecation was generally automatic. The animals seldom ate well after cervical transection. Each day they received, by stomach tube, 10 grams of dextrose dissolved in 50 cc. of milk, and occasionally they could be persuaded to eat small pieces of liver. No food was given during the twelve hours previous to the experiment.

The results of early experiments necessitated a comparison between spinal animals with intact sympathico-adrenal connections and animals in which the adrenals had been inactivated. The adrenals were inactivated by removal of the right and denervation of the left. In some of these preparations the liver also was denervated by cutting the nerves running to that organ along the hepatic artery. All animals were permitted to recover from these operations before their cords were transected.

To test the effect of central stimulation of the sciatic nerve on the sympathetic system various indicators of sympathico-adrenal activity were used. Changes in arterial pressure and heart rate were ascertained by means of a cannula attached to the left femoral artery and recorded by a mercury manometer. Samples for blood-sugar assay were obtained from a cannula inserted in the right femoral artery just distal to the saphenous branch. The Folin-Svedberg (1930) sugar-determination method for unlaked blood was used and, since the method requires but 0.1 cc. of blood, duplicate samples were always taken. The Cannon and Mendenhall (1914) procedure for measuring blood-clotting time was followed, blood being secured from the right femoral artery. When the nicitating membrane was to be used as an indicator it was sensitized by removal of the superior cervical ganglion seven days before the cord was transected. Contractions of the membrane were recorded as described by Rosenblueth and Cannon (1932).

The experimental procedure was the following. The animal was permitted to recover from the preliminary reflex activity produced by placing it on the holder and exposing and cannulating the femoral arteries, before

the direct stimulation of the nerve was undertaken. Blood samples were secured, blood-clotting time was determined, and heart rate and arterial pressure were recorded at twenty-minute intervals for an hour in order to ascertain the blood-sugar level and other conditions just before stimulation. Immediately after the last recording period the right sciatic nerve was quickly exposed and cut, and Sherrington shielded electrodes were attached to its central end. The nerve was stimulated by means of a tetanizing current from the secondary coil of an inductorium. The strength of the current was always approximately the same, being just strong enough to cause a definite twitch of the exposed muscles of the leg when the electrodes were applied directly to them. The stimulation was interrupted, being on 10 seconds, off 5 seconds, on 10 seconds, etc., for a period of three minutes. Arterial pressure and heart rate were recorded constantly throughout stimulation. Blood samples and clotting time, in addition to heart-rate and arterial-pressure records, were taken as nearly as possible at one minute, 6 minutes, 18 minutes, 30 minutes and one hour after the stimulation. Respiratory rate was recorded throughout the experiment and the rectal temperature was maintained at a normal level.

Since transection of the spinal cord rendered the animals insensitive to any manipulations which were performed below the lesions, no anesthesia was necessary in most of the experiments. When the nicitating membrane was to be used as an indicator or when any other procedure required a general anesthesia, pentobarbital sodium was administered intraperitoneally. Other details of the experimental methods will be mentioned in discussing the individual experiments and their results.

Results. The results obtained from stimulating the central end of the cut sciatic in animals with a transection of the cord at C6 are presented in tables 1, 2 and 3. There it can be seen that neither anesthesia nor decerebration with removal of the pituitary gave results any different from those obtained in the preparations which were unanesthetized and had their cranial contents intact. It is also seen that double vagotomy is without special influence. The higher basic blood-sugar levels in tables 2 and 3 were due entirely to the anesthetics and decrebration. Though the rises in blood sugar varied considerably they were all, with the exception of experiments 23 and 40, far below those obtained from animals retaining normal connections between the medulla and the spinal sympathetic outflow (Griffith, 1923; Brooks, 1931). The first blood sample after stimulation always showed some blood-sugar rise, but the second sample, that taken 6 minutes after stimulation, generally gave the highest sugar value. Occasionally the sample taken at 2 minutes after or that obtained 18 minutes after was the highest. The changes in respiratory rate, which often occurred during the period of stimulation, were never large enough to account for the observed changes in blood sugar.

Arterial pressure and heart rate were maximal during stimulation. The heart rate usually did not return to the pre-stimulation level for fifteen or twenty minutes after the end of the excitation period. The greatest pressor effects appeared during the first periods of faradization, but these frequently were followed by a drop in pressure, and the stimulations often ended with an arterial pressure 10 to 15 mm. below the initial level. After the stimulation the pressure gradually returned to normal. No attempt has as yet been made to ascertain whether this fall is due to fatigue of the vasoconstrictor mechanism or to the development of an active vasodilata-

TABLE 1

Cord transected at level of sixth cervical vertebra

No anesthetic used in these experiments.

NUMBER OF THE EXPERIMENT	NERVE STIMULATED	BLOOD SUGAR			BLOOD PRESSURE		HEART RATE	
		Blood sample before stimulation	Rise in mgm. per 100 cc.	Time at which highest level was attained in minutes after stimulation	Before stimulation in mm. of Hg	Rise in mm. of Hg	Before stimulation	Rise in beats per minute
2	Right crural	95	16	18			140	25
3	Right crural	80	16	2			150	15
4	Right crural	91	10	6			160	20
6	Right crural	97	13	2			90	10
40	Right sciatic	94	35	6	73	10	88	17
41	Right sciatic*	70	10	2	78	12	124	15
42	Right sciatic*	59	16	8	94	17	136	10
44	Right sciatic*	85	13	2	95	16	120	8
45	Right sciatic*	62	8	6	74	8	134	2
47	Right sciatic*	170	18	6	100	20	124	16
Average		90	16		85	14	126	14

* Vagi cut.

tion. The drop could not be due to vagus activity induced by the initial rises in pressure for it also occurred in animals with the vagi cut.

One other peculiar vasomotor reaction of animals with a sixth-cervical transection of the cord is worth mentioning. Unanesthetized animals with vagi intact always showed a sharp drop in arterial pressure and heart rate when the animal became excited and moved its head and neck vigorously. This vigorous activity often produced temporary syncope, and the arterial pressure and heart-rate records showed this to be due to uncompensated vagus activity. Cutting the vagi abolished the phenomenon.

Changes in blood sugar, arterial pressure and heart rate were sufficient proof that sympathico-adrenal discharge had occurred. However, con-

firmatory evidence was gained by testing the blood-clotting time and recording the reactions of the denervated nictitating membrane during and after stimulation. Thus in a few experiments five indicators of sympathi-

TABLE 2

Cord transected at level of sixth cervical vertebra

These experiments done under sodium barbital (0.3 gm. per kgm.).

NUMBER OF THE EXPERIMENT	NERVE STIMULATED	BLOOD SUGAR			BLOOD PRESSURE		HEART RATE	
		Blood sample before stimulation	Rise in mgm. per 100 cc.	Time at which highest level was attained in minutes after stimulation	Before stimulation in mm. of Hg	Rise in mm. of Hg	Before stimulation	Rise in beats per minute
10	Right sciatic	213	15	2	90	10	72	8
	Right brachial	185	10	6	150	10	96	4
11	Left crural	123	17	18	80	10	160	20
	Left brachial	105	19	6	90	30	155	25
12	Left sciatic	118	19	2			92	8
16	Right sciatic	166	9	6			120	16
	Right brachial	158	11	12			114	36
Average		152	14		102	15	115	17

TABLE 3

Cord transected at level of sixth cervical vertebra

These experiments done after decerebration under ether (pituitary removed).

NUMBER OF THE EXPERIMENT	NERVE STIMULATION	BLOOD SUGAR			BLOOD PRESSURE		HEART RATE	
		Blood sample before stimulation	Rise in mgm. per 100 cc.	Time at which highest level was attained in minutes after stimulation	Before stimulation in mm. of Hg	Rise in mm. of Hg	Before stimulation	Rise in beats per minute
22	Right sciatic	200	12	6	59	19	72	4
	Right brachial	182	14	12	44	6	76	4
23	Right sciatic	140	27	2	90	100	145	71
	Right brachial	160	25	6	100	50	148	20
26	Left sciatic	140	14	6	60	14	72	28
Average		164	18		71	37	103	25

co-adrenal activity were employed. Table 4 shows the effect of afferent stimulation upon the blood-clotting time in eight spinal preparations. The results demonstrate clearly that stimulation causes a shortening of the

clotting time. Some difficulty was encountered in obtaining a good record of the effect of central sciatic stimulation on the denervated nictitating membrane. In four cases, not invalidated by eye movements or rhythmic activity of the membrane, a slight contraction appeared towards the end of stimulation. Relaxation did not occur for three or four minutes. Figure 1 shows the nictitating membrane contractions obtained from a normal animal (*A*) and from one with a sixth-cervical transection of the

TABLE 4

Effect of central stimulation of sciatic upon blood-clotting time after transection of cord at sixth cervical level

NUMBER OF THE EXPERIMENT	TIME BEFORE STIMULATION				STIMULATION*	TIME AFTER STIMULATION			
	60′	40′	20′	2′		2′	6′	24′	60′
I. Adrenals intact									
68	4.0	3.0	4.0	7.0	*	6.0	5.0	7.0	9.0
71			7.5	8.0	*	2.5	3.5	5.0	8.0
72	5.0	5.5	5.0	5.5	*	1.5	2.5	4.5	6.0
75	8.0	5.5	7.0	5.0	*	3.0	3.0	5.5	6.0
81		6.5	6.5	6.5	*	4.0	4.0	6.0	6.5
82		10.0	10.0	14.0	*	10.0		11.0	
83		7.0	6.0	9.0	*	4.0	4.0	8.0	
85		7.0	6.5	7.0	*	4.0	5.0		8.0
Average...	6.0	6.0	7.0	8.0		4.0	4.0	7.0	7.0
II. Adrenals inactivated									
81		6.0	6.0	6.5	*	4.0	4.0	8.5	
83		4.0		5.5	*	5.5	10.0		
84	7.0	8.0	9.0	9.0	*	8.0	9.0	8.0	
85		4.0	5.0	5.0	*	4.0	3.0	4.5	5.0
86	3.0	7.0	8.0	5.0	*	3.0	3.0	7.0	6.0
	6.0		4.0	6.0	*	3.0	3.0	4.0	
87	8.0		4.5	5.5	*	6.0	4.0		6.0
Average...	6.0	6.0	6.0	6.0		5.0	5.0	6.0	6.0

cord (*C*, *C′*). Intravenous injections of comparable amounts of adrenalin (*B*, *D*) were given to test the relative sensitivity of the two membranes.

These results prove that afferent stimuli are capable of producing activity in the sympathetic system of an animal in which the higher brain centers have been disconnected from the spinal sympathetic outflow, though the activity thus produced is not as intense as that evoked by similar stimulation of normal or decerebrate animals. This indicates that afferent, presumably nociceptive impulses, passing up the cord, are cap-

able of exciting the preganglionic neurons of the sympathetic system and this in turn is physiological evidence for the existence of sympathetic connections between the tracts carrying afferent impulses up the cord and the preganglionic neurons.

It became of interest to ascertain whether this connection between afferent impulses and preganglionic neurons was made in any particular region of the cord. In five animals the cord was transected at the level of the tenth thoracic segment, thus dividing the sympathetic outflow. Table 5 presents the results obtained from stimulating an afferent nerve above the transection (the brachial) and an afferent nerve below (the

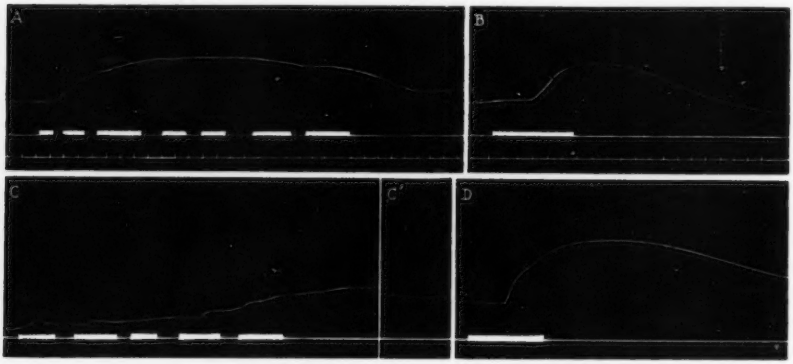

Fig. 1. *A*. Contraction of the denervated nictitating membrane in a normal anesthetized animal on stimulation of the central end of the cut sciatic nerve.

B. Effect on the nictitating membrane of the same animal of an intravenous injection of 1.5 cc. of 1:500,000 (0.5 cc. per kgm.) adrenalin solution.

C. Contraction of the nictitating membrane in an anesthetized chronic spinal preparation on stimulation of the sciatic centrally. Two minutes elapse between *C* and *C'*.

D. Effect on nictitating membrane of the same spinal animal of an intravenous injection of 1 cc. of 1:500,000 (0.5 cc. per kgm.) solution of adrenalin.

The time scale is the same for all figures—ten-second intervals.

sciatic). Stimulation of the brachial should have produced and did produce changes in blood sugar, heart rate and arterial pressure of approximately normal magnitude, for the medulla and the chief spinal sympathetic outflow were intact. Stimulation of the sciatic, on the other hand, could produce sympathico-adrenal discharge only by activating a few fibers of the splanchnics and the other preganglionic fibers having their origin in the last three thoracic and first three lumbar segments of the cord. The rise in blood sugar, heart rate and arterial pressure, produced by stimulation of the sciatic nerve centrally, clearly demonstrates that nociceptive

stimuli can activate the preganglionic sympathetic outflow in the lower as well as in the upper segments of the cord.

Griffith (1923) found that the adrenal medulla was chiefly responsible for the rise in blood sugar produced by central stimulation of a nerve in normal anesthetized cats. Tables 6 and 7 show that removal or denervation of the adrenals in spinal preparations practically abolished the rise in blood sugar and greatly reduced the changes in heart rate and arterial pressure. In some experiments (77, 79) the blood-sugar level was dropping before and at the time of stimulation, and this explains why samples

TABLE 5

Cord transected at level of tenth thoracic vertebra

These experiments performed after decerebration.

NUMBER OF THE EXPERIMENTS	NERVE STIMULATED	BLOOD SUGAR			BLOOD PRESSURE		HEART RATE	
		Blood sample before stimulation	Rise in mgm. per 100 cc.	Time in which highest level was attained in minutes after stimulation	Before stimulation in mm. of Hg	Rise in mm. of Hg	Before stimulation	Rise in beats per minute
18	Right brachial	217	45	12	68	12	224	12
19	Right brachial	250	50	12	55	9	195	20
20	Right brachial	218	22	2	60	20	175	40
24	Right brachial	257	33	6	105	115	145	85
29	Right brachial	220	93	6	100	90	204	52
Average		232	49		78	49	189	42
18	Right sciatic	238	7	2	86	9	228	7
19	Right sciatic	300	8	6	63	7	215	10
20	Right sciatic	232	15	6	80	12	170	20
24	Right sciatic	260	12	6	105	6	220	20
29	Right sciatic	190	20	3	80	10	220	10
Average		244	12		83	9	211	13

taken after stimulation give a lower sugar value than those before. Thus it cannot be said that the stimulation produced a fall in blood sugar. In other experiments (82, 87) slight rises could be similarly interpreted on the basis of a gradually rising blood-sugar level. However, in some preparations (61, 75, 81) the rise in blood sugar obtained on stimulation was large enough to be worthy of further consideration, especially since the blood-sugar level was falling at the time of stimulation. It was thought that the liver nerves might be in part responsible for the slight rises in blood sugar obtained after inactivation of the adrenals.

In five animals (table 8) the liver nerves were cut in addition to inactiva-

tion of the adrenals. Three of these gave a blood-sugar rise of a few milligrams on stimulation of the sciatic centrally. The average obtained was

TABLE 6

Cord transected at level of sixth cervical vertebra

Effect of stimulation before and after complete removal of the adrenals.

NUMBER OF THE EXPERIMENT	NERVE STIMULATED	ADRENAL REMOVAL	BLOOD SUGAR			BLOOD PRESSURE		HEART RATE		COMMENTS
			Blood sample before stimulation	Change in mgm. per 100 cc.	Time at which highest level was attained in minutes after stimulation	Before stimulation in mm. of Hg	Rise in mm. of Hg	Before stimulation	Rise in beats per minute	
16	Rt. sciatic	Before	154	16	12			118	24	Vagi intact
	Lft. sciatic	After	140	6	6			124	12	Na barbital anesthesia
75	Rt. sciatic	Before	96	17	3	136	18	132	8	Vagi cut. Pentobarbital sodium anesthesia
	Lft. sciatic	After	133	7	6	85	11	120	6	
77	Rt. sciatic	Before	51	18	18	70	12	128	8	Vagi cut. Pentobarbital sodium anesthesia. Artificial respiration
	Lft. sciatic	After	70	−3		74	6	140	2	
81	Rt. sciatic	Before	79	17	6	70	10	96	4	Vagi cut. Pentobarbital sodium anesthesia. Cocaine intravenously
	Lft. sciatic	After	132	8	6	90	22	146	28	
83	Rt. sciatic	Before	99	15	6	80	12	120	10	Vagi cut. Pentobarbital sodium anesthesia. Cocaine intravenously
	Lft. sciatic	After	156	−1		82	6	140	8	
85	Rt. sciatic	Before	55	20	18	84	14	116	12	Vagi cut. Pentobarbital sodium anesthesia. Cocaine intravenously
	Lft. sciatic	After	75	1	6	76	12	120	8	
Averages.....		Before	89	17		88	13	118	11	
		After	117	3		81	11	131	10	

lower than the average for the animals with just the adrenals inactivated, but the difference seems insignificant. Bulatao and Cannon (1925), Britton (1928), and others have shown that the liver nerves probably play

TABLE 7

Cord transected at level of sixth cervical vertebra

Adrenals inactivated.

NUMBER OF THE EXPERIMENT	NERVE STIMULATED	ADRENAL INACTIVATION	BLOOD SUGAR			BLOOD PRESSURE		HEART RATE		COMMENTS
			Blood sample before stimulation	Rise in mgm. per 100 cc.	Time at which highest level was attained in minutes after stimulation	Before stimulation in mm. of Hg	Rise in mm. of Hg	Before stimulation	Rise in beats per minute	
52	Rt. sciatic	Chronic right removed, left denervated	72	7	18	90	10	80	12	No anesthetic. Vagi intact
61	Rt. sciatic	Chronic right removed, left denervated	76	7	6	80	20	128	12	No anesthetic. Vagi intact
79	Rt. sciatic	Acute removal of both	63	−6		87	7	92	8	No anesthetic. Vagi intact
			93	−5		50	6	124	4	Vagi cut
82	Rt. sciatic	Chronic right removed, left denervated	72	2	2	72	10	108	20	No anesthetic. Vagi intact
84	Rt. sciatic	Chronic right removed, left denervated	78	6	6	74	14	128	4	No anesthetic. Vagi cut
86	Rt. sciatic	Chronic right removed, left denervated	58	8	6	66	5	136	8	Pentobarbital sodium anesthesia. Vagi cut. Cocaine and curare. Artificial respiration
	Rt. brachial		158	−3		64	6	160	0	
87	Rt. sciatic	Chronic right removed, left denervated	102	3	6	90	18	132	4	No anesthetic. Artificial respiration. Vagi cut.
			114	0		98	5	136	0	Pentobarbital sodium anesthesia curare, cocaine
Average.....			88	2		77	10	122	7	

little if any part in the discharge of liver glycogen during sympathetic activity. The results presented here would tend to confirm rather than contradict this finding.

When a rise in blood sugar was obtained after inactivation of the adrenals and denervation of the liver, it was somewhat delayed in appearance. The first sample after stimulation generally showed no rise, and if the rise appeared it was revealed by the second sample (6 minutes after) or the third (18 minutes after). Cocaine did not influence the magnitude of these blood-sugar changes.

TABLE 8

Cord transected at level of sixth cervical vertebra

Adrenals inactivated—liver nerves cut.

NUMBER OF THE EXPERIMENT	NERVE STIMULATED	BLOOD SUGAR			BLOOD PRESSURE		HEART RATE		COMMENTS
		Blood sample before stimulation	Change in mgm. per 100 cc.	Time at which highest level was attained in minutes after stimulation	Before stimulation in mm. of Hg	Rise in mm. of Hg	Before stimulation	Rise in beats per minute	
43	Rt. sciatic	105	−4		43	5	124	4	Ether while vagi were cut
76	Rt. sciatic	62	3	6	74	7	104	8	Pentobarbital sodium anesthesia. Vagi intact. Cocaine intravenously
78	Rt. sciatic	61	1	6	76	10	112	4	No anesthetic. Vagi cut
	Lft. sciatic	84	−4		72	4	112	4	Artificial respiration
89	Rt. sciatic	86	1	12	86	4	140	0	Pentobarbital sodium anesthesia
	Rt. sciatic	105	7	12	80	8	136	4	Vagi cut. Cocaine
91	Rt. sciatic	107	4	6	66	4	104	8	No anesthetic
	Rt. sciatic	108	1	1	68	6	104	4	Vagi cut
Average.....		90	1		71	6	117	5	

Cannon and Mendenhall (1914) found that removal of the adrenals abolished the effect of splanchnic stimulation on blood-clotting time. The second part of table 4 gives the results which were obtained in a study of the blood-clotting time in spinal animals with adrenals inactivated. A slight acceleration of the clotting was recorded in some experiments by sciatic stimulation, but the average change was scarcely significant. The same type of result was obtained after liver nerves were cut in addition to inactivation of the adrenals. No contraction of the denervated nictitating membrane could be elicited by afferent stimulation of the spinal animal

after removal of the adrenals, even though large doses of cocaine were used to further sensitize the membrane.

It is known (Langley, 1900) that in the cat the sympathetic outflow from the cord does not extend below the third lumbar segment. A transection of the cord caudal to that level would eliminate the possibility of any reflex excitation of the sympathetic nerves on stimulation of the central end of the cut sciatic. Muscular reflexes, however, would be produced, and

TABLE 9

Cord transected at level of third lumbar vertebra

NUMBER OF THE EXPERIMENT	NERVE STIMULATED	BLOOD SUGAR			BLOOD PRESSURE		HEART RATE	
		Blood sample before stimulation	Change in mgm. per 100 cc.	Time at which highest level was attained in minutes after stimulation	Before stimulation in mm. of Hg	Rise in mm. of Hg	Before stimulation	Rise in beats per minute
13	Right brachial†	80	29	6	110	30	190	7
14	Right brachial†	184	32	6	95	42	184	24
25	Right brachial†	91	45	2	114	80	150	54
27	Right brachial†	286	30	2	90	48	192	64
90	Right brachial*	67	20	12	120	48	160	32
92	Right brachial*	81	24	18	84	26	192	16
93	Right brachial*	50	29	12	100	26	216	20
Average		120	30		102	43	183	31
13	Right sciatic†	152	−6		126	4	240	5
14	Right sciatic†	190	−4		94	4	184	−4
21	Right sciatic†	277	−7		46	4	96	4
25	Right sciatic†	118	−6		110	8	148	6
27	Right sciatic†	286	4	6	128	4	256	8
90	Right sciatic*	55	−1		96	−10	184	−12
92	Right sciatic*	90	2	1	102	0	176	0
93	Right sciatic*	66	−6		120		220	4
Average		154	−3		103	2	188	1

† Experiments performed after decerebration.

* Experiments performed under pentobarbital sodium.

if muscular metabolites were responsible for the rise in blood sugar obtained after inactivation of the adrenals, it should still be obtained. Eight experiments of this type were performed (table 9). The animals were all in especially good condition and they had been kept a week after lumbar transection. Three were given pentobarbital sodium and five were etherized and then decerebrated. Stimulation of the brachial nerve elicited practically normal changes in blood sugar, arterial pressure, heart rate and

blood-clotting time. Stimulation of the sciatic produced no significant changes in any of these.

Discussion. The fact that nociceptive stimuli can produce activity of the spinal sympathetic outflow in the absence of higher centers reveals the presence of a spinal mechanism making possible reflex excitation of the preganglionic neurons of the sympathico-adrenal system. Stewart and Rogoff (1917) stated that activity of the adrenal glands was controlled by a center located in the cervical region of the cord but that this center was not subject to control by higher centers nor was it influenced by painful stimuli. The observations presented in this paper tend to show that if any mechanism controlling the adrenal glands is present in the cord it is a reflex mechanism. The changes in blood sugar, arterial pressure and heart rate were, at the most, less than half as great as those obtained from anesthetized normal and unanesthetized decerebrate animals by similar stimuli. The methods used were not sufficiently accurate to support any exact conclusions concerning the quantitative side of the problem. This work, however, furnishes additional evidence that the centers chiefly responsible for normal reflex excitation of the sympathico-adrenal system are located in the medulla or some higher region of the brain.

Two possible objections to the above conclusions are, that the spinal cats could not be properly nourished and that recovery from spinal shock was not complete. The nutritional state of the spinal preparations was not normal, though the animals were given some carbohydrates. On the other hand, it was not possible to obtain from the spinal preparation reflex activity of the sympathico-adrenal system as great as that which could be produced from equally poorly nourished normal animals. It is also true that two weeks may not be a sufficiently long period to permit complete recovery from spinal shock. The work of Richter and Shaw (1930), concerning the effect of transections of the cord on sweating, perhaps indicates that the period of greatest activity of the cord does not occur until about the fourth week. However, at the time of stimulation, in the present experiments, the cord was very active, and vigorous crossed-extension and flexion reflexes could be elicited. Merely turning the animals over often produced violent simultaneous extension, flexion or kicking of the hind legs.

The rises in blood sugar obtained after inactivation of the adrenals and denervation of the liver were almost within the range of the experimental error of the method. In these animals the sympathetic cardio-accelerator and vasoconstrictor nerves were active, for rises in heart rate and arterial pressure appeared immediately on stimulation. Since sympathin (Newton, Zwemer and Cannon, 1931; Cannon and Bacq, 1931) would be produced by this activity and since this hormone has many of the same effects as adrenin, the slight rises in blood sugar might be due to sympathin.

Bacq (1933) was able to obtain rises in blood sugar which he thought must be due to sympathin. Until a possible direct effect of sympathetic nerves upon muscle glycogen has been ruled out, I do not feel that the matter can be completely settled.

SUMMARY

Rises in blood sugar, increments in arterial pressure and heart rate, diminished clotting time, and contraction of the denervated nictitating membrane can be reflexly induced from the spinal cord below the sixth-cervical segment.

The observed activity implies the presence of a spinal mechanism whereby the preganglionic neurons of the sympathetic system can be activated directly by nociceptive impulses traveling up the cord. This excitation of the preganglionic neurons can occur in the lower as well as in the upper thoracic region of the cord.

The sympathetic discharge which can be reflexly elicited from the isolated spinal cord is not as intense as that which the same stimuli produce when the medulla and higher centers retain their normal connections with the spinal sympathetic outflow.

Transections of the cord caudal to the sympathetic outflow abolish the effects elicited by stimulation of the sciatic centrally, when the transection is higher.

Inactivation of the adrenals and denervation of the liver greatly reduce but do not completely abolish the rises in blood sugar, heart rate and arterial pressure.

I wish to thank Prof. Philip Bard and Prof. W. B. Cannon for the assistance and suggestions I have received.

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THE INTERRELATION OF A GONOTROPIC HORMONE AND VITAMIN A[1]

S. B. D. ABERLE

From the Department of Obstetrics and Gynecology, Yale University School of Medicine

Received for publication July 5, 1933

Hormones and vitamins have specific effects on the organism. The absence of vitamin-A in the diet of albino rats causes a cornification of epithelial tissue in the body. A gonotropic hormone produces cells in the vaginas of spayed animals which are similar to those found in pregnancy. Both of these substances affect epithelial tissue in a definite manner, but in each instance a different cell-type is produced. Questions arise as to the interaction of the two—i.e., whether vitamin A and a gonotropic hormone each act directly and without regard to the presence or absence of the other; or whether a gonotropic hormone can produce its characteristic action in an environment altered by the absence of vitamin A. In an effort to try to answer these questions rats suffering from A-avitaminosis were injected with a hormone standardized for the production of pregnancy-like vaginal cells.

That the cells of the vagina of rats undergo a definite histological change during pregnancy has been shown by Florey (3). Their metabolism is so changed that instead of manufacturing eleidin the mucosa elaborates mucin. The mucin droplets are formed in that part of the cells occupied by the Golgi body. There is an increase of the Golgi substance, an increase in the size of the protoplasm, and an alteration of nuclear shape. It is in no sense a degeneration of the protoplasm, as the mucus-producing cells present features exhibited by glandular cells, such as those in the colon. Kelly (7) has demonstrated mucoid changes in the vagina of the guinea pig during pregnancy. Mirskaia and Wiesner (9) have shown that mucification occurs regularly prior to the first oestrus in the mouse. The production of the mucoid type of cell in rodents' vaginas has been observed under the stimulation of corpora lutea hormone (4, 5, 8, 11) and of follicular fluid (2) and from extracts of the human placenta.

[1] This investigation was partially subsidized by a grant from the National Research Council Committee for Research in Problems of Sex. The term *gonotropic* is here used in the sense of stimulating the genital tract or part of it directly and not through the ovaries, as implied in the term *gonadotropic*.

METHOD. The material used in this study was obtained from human placentas. The placentas were ground up and the tissue allowed to stand in three times its bulk of 95 per cent alcohol for not less than ten hours. It was then filtered and the solid part divided into portions of approximately 250 grams each. Hot alcohol (1.1 cc.) was used for every gram of tissue. It was percolated for three one-hour periods, fresh alcohol being used at the end of each hour. The tissue was then washed with a small amount of ether. The percolated extracts were filtered, mixed with the alcohol in which the tissue was dehydrated, and the whole taken to dryness by distillation on a boiling water bath. The dry residue was purified by extraction with ether, filtration and distillation. The concentrated ether-extract was transferred to a weighed evaporating dish and taken to dryness in a vacuum desiccator. The final product was a gummy residue. The residue was made into an aqueous solution and assayed. Three injections of 1.5 mgm. each of the gum were found to produce cornification in the vaginas of spayed female rats. Five-tenths milligram of the gum in each of 3 injections produced mucoid vaginal cells in mice. In order to determine the time of their appearance and duration, seventeen mice were injected and killed at 24, 36, 48, 60, 72 and 84 hours. The vaginas were sectioned and studied. It was found that mucoid cells appeared at 24 hours after the first injection and were still present at 60 hours. After that, cornified cells made their appearance. Five spayed rats were then injected with 0.9 mgm. of the gum and smears taken at four-hour intervals. The pseudopregnancy cells appeared and disappeared at the same time as in the mouse.

The experimental rats were obtained from a stock in which the care and feeding had been carefully standardized. Breeding females were fed 97 per cent calf-meal[2] and 3 per cent cod liver oil until the 13th day of lactation, when they were put on 100 per cent calf-meal until the young were weaned. All litters were reduced to six on the day of parturition and weaned at 21 days.

A total of twenty-five rats commenced the experiment. Three died before the experiment was terminated. The data on the remainder are shown in table 1.

The composition of the vitamin-A-deficient diet was:

INGREDIENT	PER CENT
Casein[3]	18
Cornstarch	56
Hydrogenated vegetable oil[4]	22
Osborn-Mendel salt mixture IV	4

[2] Calf-meal is a commercial mixture containing approximately the following ingredients in 100 parts: oil meal 15, malted barley 10, whole wheat 22, oat flour 15, dried skim milk 15, yellow corn meal 20, ground limestone 1, steamed bone meal 1, salt 1.

[3] Eimer and Amend technical casein.

[4] Crisco.

TABLE 1

Ovariectomized rats injected with placental hormone

	WEANED	OVARIECTOMIZED	CORNIFIED VAGINAL SMEAR	XEROPHTHALMIA	WEIGHT-GAIN STOPPED	INJECTIONS,* 1ST SERIES	REACTION	INJECTIONS,† 2ND SERIES	REACTION	INJECTIONS,† 3RD SERIES	REACTION	INJECTIONS,† 4TH SERIES	REACTION	KILLED
							hours		*hours*		*hours*		*hours*	
Group I. Rats on purified diet deficient in vitamin A:														
Number rats	12	12	12	8	9	9		4		3		2		12
Mean age	21.3	59.6	59.7	66.3	65.9	72.3		68.5		73.3		85.0		77.0
S.D.M.	0.1	1.2	3.1	3.6	1.9	2.3								2.0
Mean weight‡	37.7	115.9	117.5	126.0	124.6	124.7		130.5		130.3		128.0		122.8
S.D.M.	1.2	6.3	3.0	7.6	5.7	7.5								4.3
Group II. Control rats on purified diet with cod liver oil:														
Number rats	5	5				2	2	4	4	2	2	1	1	5
Mean age	21.4	47.6				61.0	42.0§	71.5	46.5§	70.5	43.0§	84.0	50.0§	75.6
S.D.M.	0.2	3.2												4.4
Mean weight‡	33.8	120.4				163.5		207.5		184.5		167.0		205.6
S.D.M.	1.6	12.9												16.4
Group III. Control rats on table-scrap diet:														
Number rats	5	5				5	5							5
Mean age	21.0	52.8				73.0	39.0§							74.6
S.D.M.	0.0	0.9				5.1	2.4							5.4

* Three injections of 0.9 mgm. each at approximately six-hour intervals.

† Six injections of 0.9 mgm. each at approximately six-hour intervals.

‡ Weight on nearest day ± 1 to 4 days.

§ Hours after the first injection.

Adequate amounts of vitamins B, D and E were supplied by yeast,[5] irradiation,[6] and hydrogenated vegetable oil. Vitamin C has not been found necessary in the normal metabolism of the rat (10). The tests of the yeast showed that one-tenth of a gram daily was sufficient to produce a gain in weight in rats deprived of vitamin B. The same batch of yeast was used throughout the experiment. The animals received 0.4 to 0.8 gram of 1:1 irradiated and non-irradiated yeast daily. To irradiate the yeast it was spread in thin layers, exposed to the rays of a mercury vapor lamp at a distance of 15 inches for 15 minutes, then mixed, respread and irradiated for another 15 minutes (6). A biological test of the ergosterol used showed that 0.0009 mgm. fed over a period of ten days gives a two-plus line-test; 0.001 to 0.002 mgm. were given daily.

Seventeen rats were put on the purified diet, twelve rats were kept without additional vitamin A, five were given cod liver oil as controls. Five were kept on a table-scrap diet (table 1). All of the 22 rats were ovariectomized between the ages of 39 to 66 days. Following ovariectomy smears were taken on the control rats until they had exhibited a scant dioestrous smear for five days. The rats on the A-deficient diet had smears taken until they exhibited cornified vaginal cells. This occurred at a mean age of 59.7 days, weight 117.5 grams. Continual cornified vaginal cells have been found to be the most delicate index of avitaminosis of A in both normal and ovariectomized rats, and once established persists as long as the deficiency exists (1).

Eight rats on the purified diet without vitamin A showed xerophthalmia at a mean age of 66.3 days, weight 126.0 grams; seven stopped gaining weight before the injections were given. Sixteen rats including nine A-deficient animals and seven controls were injected with 0.9 gram of the gummy residue containing the hormone (table 1). At from 36 to 43 hours after the first injection eight rats on the A-deficient diet, one on the purified control diet and all the rats on the table scrap diet were killed. The vaginas were fixed and sectioned. One A-deficient and one control were followed for a week with smears at 8, 12, 4 and 8 o'clock each day. It was thought that possibly in this way a delayed reaction might be detected. None of the sections of the vaginas of the A-deficient animals showed any mucoid cells; the vaginas were completely cornified. No mucoid epithelial cells were detected in the smears. All the animals on the control diets showed mucification of the peripheral vaginal cells and the smears showed similar pictures.

The amount of hormone was then increased. Four A-deficient rats and four control rats on purified diet with cod liver oil were given twice the amount of hormone found necessary to produce mucification in the con-

[5] M. H. Givens of the Northwestern Yeast Company supplied tested yeast.

[6] Irradiated ergosterol was supplied by the Fleischmann laboratories.

trols of the first group. One A-deficient animal and two controls were killed for histological study and smears of the remaining animals were taken at intervals, as above, for a week. Again, A-deficient rats showed no mucification in histological sections or in the smears, while the controls showed the characteristic reaction.

At the end of a week three A-deficient rats and two controls were again given six injections of the hormone, since there was a possibility that from the previous injections their vaginas might be in a more susceptible state. One A-deficient rat and one control were killed at the end of 43 hours and the vaginal contents of the remaining animals watched for another week. The vagina in the control showed mucification but only cornification was present in the A-deficient animal. The remaining three animals were again injected after a week with the double dose of hormone and killed at the end of 50 hours. The vaginal epithelium of the control and experimental animals showed the same reactions as before. In none of the A-deficient rats were pregnancy-like cells found, either in the smears or in the histological sections. In all of the control animals pregnancy-like cells were found both in smears and in histological sections of the vagina. In two control animals cornification had commenced and the mucoid cells were in the lumen of the vagina.

The mitotic figures were counted in the vaginas of four groups of rats to find whether or not the cells of the vagina of the A-deficient animal were viable and capable of proliferation. In each animal all the mitotic figures in five complete cross-sections taken at comparable places in the vaginas, were counted. The results were as follows:

NUMBER OF ANIMALS	MEAN NUMBER OF MITOTIC FIGURES IN EACH SECTION
4 non-injected; non-ovariectomized table-scrap diet at pre-oestrus ..	13.2
4 non-injected, ovariectomized, A-deficient diet	20.7
4 injected, ovariectomized, purified diet with cod liver oil	39.1
4 injected, ovariectomized, A-deficient diet	11.6

The mitotic figures in the A-deficient non-injected rats were almost twice as great as the mitotic figures in the ovariectomized, injected rats. The response in the A-deficient injected animals was not as great as that in animals given adequate vitamin A, but it compared well with the activity of the vaginal cells of the non-ovariectomized rats at pre-oestrus.

As a result of the injections the uteri of five control ovariectomized rats on the purified diet with cod liver oil showed one animal in which the distension of the lumen was as great as that found at oestrus, and one animal where the distension was slightly less. In the three remaining

animals the uteri were the size usually found in the castrate animal. Of the twelve uteri of the A-deficient injected animals, seven were no larger than those usually found in the castrate rat, three showed some distension of the lumen, and in two the distension was as great as that found at oestrus. The lack of distension in some of the uteri was probably due to the fact that the animals were not allowed to live a sufficient time to show the maximal response. In two rats areas of the uterine epithelium were cornified, and the lumen was filled with desquamated cornified cells.

CONCLUSIONS

A placental extract standardized for its production of mucoid vaginal cells was incapable of producing any effect on the vaginal cells of rats suffering from avitaminosis of A.

The mitotic figures present in the vaginas of rats on the A-deficient diet showed that the cells were viable and capable of proliferation. That the hormone was effective in the A-deficient rats was shown by the distention of some of their uteri.

The control animals kept under identical environmental conditions and fed the same purified diet except for the addition of vitamin A invariably responded to the hormone by the production of mucoid vaginal cells. This shows that avitaminosis of A was responsible for preventing the characteristic action of a hormone.

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SOME ATYPICAL RESPONSES OF RABBITS TO INSULIN

ISOLDE T. ZECKWER

From the Department of Pathology, University of Pennsylvania Medical School, Philadelphia, Pa.

Received for publication July 1, 1933

This paper concerns the response of a series of 40 rabbits to insulin, among which 6 rabbits were found to tolerate without convulsions insulin in much larger doses than the average dose which caused convulsions in all the others, and 3 showed slight resistance. These resistant animals are described, first because of the practical importance in recognizing the degree of resistance and its frequency of incidence when any experiments on insulin action are carried out on rabbits, and secondly, because of the theoretical interest in attempting to elucidate what factors are responsible for this abnormal resistance to the action of insulin. This study is the continuation of a brief preliminary report of the earlier experiments in this series of rabbits (1).

METHODS. Rabbits of different breeds and both sexes were used. Insulin was injected subcutaneously by means of sterile dry tuberculin syringes. When small doses were used U-10 insulin (Lilly) was diluted so as to make up solutions containing 1, 2, or 5 units to each cubic centimeter. When larger doses were given, U-10, U-20, and U-40 insulin were used. The rabbits were fasted 16 to 22 hours. Scott (2) has shown from a large number of determinations that the blood sugar of rabbits reaches its lowest point at about 10 hours' fast and is approximately constant from then to 24 hours. The slight variation in the fasting period in a herbivorous animal was, therefore, not considered important. Blood sugar determinations were carried out by the Hagedorn and Jensen method on blood collected from the marginal ear vein while the rabbit was in its natural unrestrained position. When convulsions occurred the animals were immediately resuscitated by injecting glucose solution intravenously. The rabbits were under observation from 5 to 8 hours before recording absence of convulsions. They were all fed on similar diets of hay, oats and fresh greens. Sufficient time was allowed to elapse between consecutive insulin injections to permit the resumption of the regular diet. Injections were usually made once or twice a week.

EXPERIMENTS. *Tolerance to insulin under varying conditions.* The first 2 resistant rabbits encountered (see table 1) were R 52 which withstood

20 units of insulin without developing convulsions but on a later date convulsed with 40 units; and R 56 which withstood 66 units, but later convulsed with 76 units.

Sahyun and Blatherwick (3) had found that rabbits not accustomed to insulin convulsions are more resistant to insulin than those which had been "educated" by experiencing convulsions several times. They found in 2 of their rabbits that an "immunity" developed by repeatedly injecting them with ascending sub-convulsive doses until 50 units per kgm. body weight resulted in no convulsions. They do not state among how many rabbits this phenomenon was noted. The same authors later (4) induced this "immunity" artificially in the following manner. They took 3 males and 3 females on a high carbohydrate diet and gave them gradually ascending non-convulsive doses until these rabbits withstood 82, 90, 100, 90 and 85 units respectively. The authors avowed no intention of explaining this phenomenon.

In view of these findings of Sahyun and Blatherwick, a review of the history of R 52 and R 56 is important. With R 52 convulsions had developed with 3 units, following which the dosage had been increased as follows: 3.5, 4, 5, 5, 7, 10, 20 units covering a period of 8 weeks, without convulsions. R 56 had received the following successive doses: 2, 3, 4, 5, 6, 7, 10, 20, 40, 50, 60, 66 units without convulsions, covering a period of 9 weeks. It therefore appeared possible that this "immunity," or more properly, refractoriness or resistance was a gradual development resulting from ascending non-convulsive doses. Therefore to test this, 13 rabbits were subjected to very slowly ascending doses with especial care to begin with doses of 1.5 unit and increase very gradually, sometimes increasing only 0.25 or 0.5 unit at a time and covering a period of many weeks. The results showed no 100 per cent "immunity" development, such as Sahyun and Blatherwick were able to produce, but complete inability of 10 rabbits to tolerate without convulsions 1.75 , 1.5, 1.5, 1.5, 2, 4.75, 2, 1.75, 1.25, and 3.5 units respectively, while 3 rabbits tolerated increased dosage without convulsions as follows: R 82, 24 units; R 85, 9 units; R 88, 10 units.

Several of the resistant rabbits were subjected to convulsions to see if when "educated" to convulsions, in the sense of Sahyun and Blatherwick, they would then become responsive to the usual doses. When thus treated to gradually descending convulsive doses, R 52, subjected to convulsions 5 times during a period of 3 weeks, convulsed with 20, but not with 15 units 2 days later, and R 82, subjected to convulsions 14 times during 14 weeks, finally convulsed with 3 units 2 days after the previous convulsion. It seems probable that this "education" to convulsions really means that each dose that is convulsive results in such a degree of hypoglycemia that the compensating glycogenolysis which is known to occur, eventually reduces the liver glycogen to the point where even with intervening feed-

ing the glycogen store has fallen low. In consequence less compensating glycogenolysis can occur on the next insulin injection, and the insulin being unopposed by sufficient glycogenolysis results in a convulsive level being reached more readily. Yet even so, R 52 just after having been subjected to convulsions 5 times within 3 weeks still tolerated without convulsions 15 units.

Similarly table 1 shows that R 85 and R 88 convulsed with smaller doses when repeated convulsive descending dosage was given than when the dosage was ascending.

In order to gain light on whether the refractoriness was really acquired or whether it might be spontaneous, 2 of the rabbits, the quite resistant R 82 and the very slightly resistant R 89, were left uninjected for over 3 months. It was assumed that any effect of often repeated sub-convulsive doses would have been lost during that period, and furthermore these rabbits had been subjected to convulsions before this rest period so that this refractoriness had been proved to have been broken down to some extent. After this rest period in order to gauge their natural refractoriness they were each injected at once with 4 units, a dosage which had resulted in convulsions in all the non-refractory rabbits of this series of 32. Neither rabbit developed convulsions. R 89 which had been only slightly refractory before the rest period, succumbed to its second injection after the rest period with the same dose that had been required to produce convulsions previous to the rest period. R 82 a week later tolerated 8 units, but after another 10 days convulsed with 20 units, only, however, after an abnormally long interval of 5 hours following the injection. This then indicates that this rabbit had a high degree of natural refractoriness, which could be increased again by being injected repeatedly with non-convulsive doses to the point of tolerating 40 units.

These observations and the fact that 10 rabbits out of 13 could not be made refractory indicated that a certain natural refractoriness is a requirement at the outset before any greater tolerance could be developed by repeated sub-convulsive doses.

Blood sugar curves following insulin. Figure 1 shows superimposed the blood sugar curves following non-convulsive doses of insulin in the refractory rabbits R 52 and R 56 and the normal controls R 77 and convulsive dose in normal rabbit R 31. The curve in the control rabbit R 77 is after the maximum dose of insulin that was tolerated in this animal without convulsions, the same dose having on another occasion resulted in convulsions. This, therefore, seems a fair comparison with the refractory rabbits, which though receiving much larger absolute doses were still not beyond the dose which could be tolerated by those rabbits without convulsions. In Sahyun and Blatherwick's experiments the blood sugar began to rise at about the third hour. It will be seen from figure 1 that the hypogly-

cemic period lasted a longer time in the refractory rabbits R 52 and R 56 than in the normal. The possible significance of this will be discussed later.

Drabkin and Schilkret (5) have found that if dogs were dehydrated by depriving them of water, doses of insulin which were convulsive under normal conditions did not cause convulsions, but the animals showed profound hypoglycemia and coma, and died even if glucose was given, so that

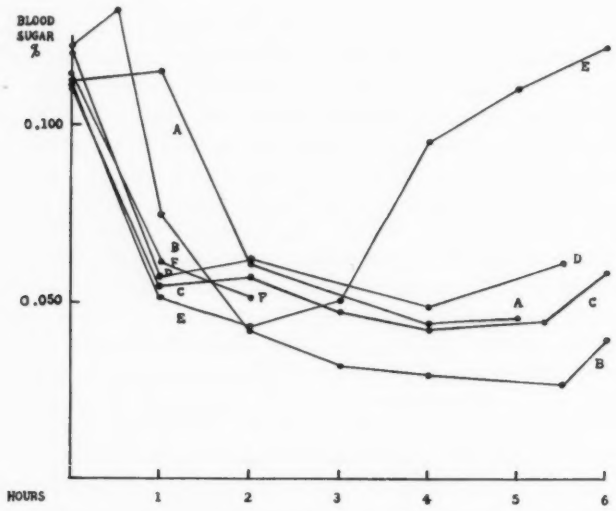

Fig. 1. Blood sugar curves in four rabbits after varying doses of insulin.

A—R 56, Feb. 11, 66 units. Resistant rabbit. No convulsions.
B— 56, Feb. 20, 20 units. Resistant rabbit. No convulsions.
C—R 52, Feb. 13, 15 units. Resistant rabbit. No convulsions.
D— 52, Feb. 20, 10 units. Resistant rabbit. No convulsions.
E—R 77, June 23, 4 units. Normal non-resistant rabbit. No convulsions.
F—R 31, June 4, 3 units. Normal non-resistant rabbit. Convulsions occurred several minutes after last blood sugar sample.

these dehydrated dogs were considered more susceptible to insulin. The rabbits reported in the present experiments did not present this picture. When they did not develop convulsions they showed slight or no hypoglycemic symptoms and required no glucose administration for recovery.

There are certain factors which have been reported to influence the response of rabbits to insulin, such as the color and breed of the rabbits. Acton and Bose (6) in India noted that certain Himalayan rabbits were not as sensitive to insulin as Belgian hares. Sahyun and Blatherwick (3)

state that white rabbits are very resistant. Ssargin (7) noted that albinos and long-haired rabbits, irrespective of color, showed resistance. In the experiments reported in the present paper, the resistant rabbits were of various types, the only white rabbit, however, R 56, being the most resistant of the series. Diet is considered to play an important part in the response of rabbits to insulin (Blatherwick, Long, Bell, Maxwell and Hill (8), Page (9), Geiger and Kropf (10) and others). Ssargin (7) has recently reviewed some of the literature on these points. All the rabbits reported in this paper were on the same diet, hay, oats and greens.

Aside from the above mentioned factors, certain other physiological factors must be considered.

Effect of thyroidectomy. It has been shown by a number of investigators that after thyroidectomy in a large variety of animals convulsions develop with much smaller doses of insulin than that required to cause convulsions before thyroidectomy. Reference should be made to Britton and Myers (11) for a review of the literature on this subject.

Four of the refractory rabbits and 4 of the normal controls were thyroidectomized aseptically and their response to insulin studied for a period of a month or more following operation. They became gradually more responsive to insulin (see table 1).

The data of normal rabbits given by Burn and Marks (12) indicate that in their experiments insulin became from 3 to 6 times (probably 9 times) more effective after thyroidectomy. It will be seen from table 1 that in the case of the refractory rabbits R 52 and R 56 there was an increase of sensitiveness to insulin after thyroidectomy varying from 10 fold to 20 fold, while the control non-refractory rabbits showed an increase in sensitiveness varying from 2.5 to 4.6 fold, while the very slightly resistant rabbits R 89 and R 102 responded much like the non-refractory ones. This seemed to indicate that the removal of the thyroid had much more pronounced effect on the markedly refractory rabbits than on the non-refractory controls. On the other hand the thyroid alone is not responsible for the resistance, as none of the refractory animals showed after thyroidectomy the sensitiveness to the minute doses of insulin that the normal rabbits showed. There must, therefore, be other factors concerned in this spontaneous resistance to insulin.

Weights of organs and their histology. Since experimentally and clinically, carbohydrate metabolism may be altered by the activity of endocrine organs other than the thyroid, the pituitary, adrenals, and other organs of R 52 were weighed and found to differ in no significant way from the standards for normal in rabbits established by Brown, Pearce and Van Allen (13). Grossly and microscopically the organs showed no pathological changes. All thyroids removed from resistant and normal rabbits were carefully examined and showed no gross or microscopic deviation from physiological variations.

TABLE 1

REFRACTORY RABBITS	LARGEST DOSE OF INSULIN (IN UNITS) TOLERATED WITHOUT CONVULSIONS	SMALLEST DOSE OF INSULIN (IN UNITS) USED WHICH RESULTED IN CONVULSIONS		INCREASE IN SENSITIVENESS AFTER THYROIDECTOMY
	Before thyroidectomy	Before thyroidectomy	After thyroidectomy	
R 52	20 A. D. 15 D. D.	40 A. D. 20 D. D.	2 (29 days post-operative)	20 × to 10 ×
R 56	66 A. D.	76 A. D.	5 (17 days post-operative)	14 × to 15 ×
R 82	24 A. D. 15 D. D. after rest period 40 A. D.	28 A. D. 3 D. D. 20 A. D. after rest period 60 A. D.*		
R 85	9 A. D.	12 A. D. 8 D. D.*		
R 88	10 A. D.	12 A. D. 8 D. D.*		
R 103	15 A. D. 5 D. D.	8 D. D.		
SLIGHTLY REFRACTORY RABBITS				
R 89	4.5 A. D.	4.75 A. D. 3.5 D. D.	3 (9 days post-operative)	1.5 ×
R 98	5.5 D. D.	6 D. D.		
R 102	4	6 D. D.	2 (1 month post-operative)	3 × to 2 ×
NON-REFRACTORY CONTROL RABBITS				
R 54	3	3.5	0.75 (18 days post-operative)	4.6 ×
R 75	2.5	3	0.75 (30 days) post-operative)	4 ×
R 77	4	4	1 (22 days post-operative)	4 ×
R 101	2	2.5	1	2.5 ×
Average of 27 other non-refractory rabbits		2		

* Died in hypoglycemia before lower doses were tried.
A.D. = ascending dosage.
D.D. = descending dosage.

Discussion. Chemical analyses of the glycogen in the liver of these animals were not carried out because it was considered that the taking of biopsies during the course of the experiments would have introduced un-

desirable complications that would have prevented study of the points desired. However, certain speculations concerning the compensating glycogenolysis which probably occurred in these animals, though unproved by actual analyses, seem logical deductions and may serve to bring the results of these experiments in line with other types of experiments reported in the literature.

It has been shown by numerous investigators that when the blood sugar falls to a low level after insulin, glycogen is discharged from the liver and tends to arrest the decline in blood sugar, and permits spontaneous recovery unless the dose of insulin is too large. This compensating discharge of glycogen from the liver has been shown by numerous investigators to be determined by activity of the sympathetic nervous system and adrenals. The degree with which glycogenolysis can offset convulsions depends upon how much glycogen is available and upon how readily it can be mobilized by activity of the sympathetic nervous system and adrenals. Animals in which the adrenals have been inactivated by operation develop convulsions with much smaller doses of insulin than the same animals did before operation (Britton, Geiling, and Calvery (14) and others). In previous experiments I (15) found that rabbits for long periods after removing one adrenal and denervating the other will convulse with as little as 0.5 unit per rabbit. Dworkin (16) found extreme sensitiveness to insulin in sympathectomized cats.

From this point of view, looking at the curves of figure 1, we can conjecture that in the resistant rabbits glycogen is more readily released so that instead of a downward fall terminating in convulsions in 2 hours as in a normal rabbit (curve *F*), the effect of insulin is counteracted for many hours with final restoration of the blood sugar to normal without the introduction of new glucose. It is known that in rabbits which have been deglycogenated, recovery of the blood sugar is much delayed. The failure of a rapid return rise of blood sugar within 6 hours in the two resistant rabbits (curves *A*, *B*, *C*, *D*) as compared to the normal recovery with nonconvulsant doses (curve *E*) suggests that the resistant animals had lost much glycogen in arresting convulsions.

Marks (17) who is one of the few to discuss the subject of refractory rabbits says that rabbits may be abnormally refractory in 2 ways: *1*, they may show very little fall in blood sugar after insulin and this he speaks of as "metabolic insensitiveness," or *2*, the blood sugar may fall but the animal show no symptoms, and this he speaks of as "nervous insensitiveness," and states that this type can "apparently be developed to some extent by habituation." Laqueur and de Jongh (18) in a study of insulin injections in 136 rabbits, lists 9 rabbits as relatively insensitive. Their table shows that this insensitiveness consists in the blood sugar not being lowered as much as in other rabbits and a failure of the development of convulsions in

5 of the rabbits with doses of 4.5, 6, 7.5, 9, and 9 units respectively. These rabbits had on other occasions reacted normally. The units indicated are of European insulin of the year 1925. The term "nervous insensitiveness" to insulin might be applied to the resistant rabbits reported in the present paper if by that is understood absence of convulsions after insulin which probably is due to increased activity of the sympathetic nervous system in inducing effective glycogenolysis. The abrupt fall in blood sugar in figure 1 indicates that in these rabbits there was no chemical neutralization of insulin or "metabolic insensitiveness."

The relation of the thyroid to insulin action is probably by way of the adrenals and sympathetic nervous system. Burn and Marks (12) have shown that when thyroid is administered to rabbits, at first larger doses of insulin are tolerated than normally, because apparently the sympathetic nervous system is more sensitive in hyperthyroidism, and a consequently more ready glycogenolysis counteracts the insulin effect. Later when thyroid feeding was pushed to the point of depleting liver glycogen, the rabbits became abnormally sensitive to insulin, as sufficient glycogen was not available to counteract insulin. Burn and Marks showed that adrenaline was more effective in inducing hyperglycemia in thyroid-fed rabbits so long as the glycogen reserve of the liver was maintained, while thyroidectomized rabbits were less responsive to adrenaline. In other words, an excess of thyroid hormone favors glycogenolysis by adrenaline, or indirectly by insulin, while a decrease inhibits glycogenolysis. The same authors found that the animals most responsive to adrenaline were least responsive to insulin and vice versa, which constitutes further evidence that the sensitiveness of the response of the sympathetic nervous system is an important factor in determining the response to large doses of insulin. It should be emphasized that all experiments reported in the literature describing increased response to insulin after thyroidectomy have been concerned with doses of insulin so large as to evoke glycogenolysis. In some experiments in progress at present I have so far found no evidence that thyroidectomy has any effect on the blood sugar lowering action of insulin when small doses of insulin are used at a high blood sugar level in depancreatized dogs, the insulin dosage being such that the fall in blood sugar would not be sufficient to evoke glycogenolysis. All the foregoing evidence seems to indicate that the removal of the thyroid, which invariably increases the sensitiveness of various animals to convulsant doses of insulin, may operate by lessening the sensitiveness of the sympathetic nervous system, and in consequence, though abundant glycogen is present in the liver, it is not released sufficiently to prevent convulsions after large doses of insulin.

The data on the effect of thyroidectomy on insulin action bear out the conclusions in regard to the effect of thyroidectomy on glycogenolysis induced by other agents. When killed bacteria of various types are injected

intravenously into rabbits there results an immediate hyperglycemia (Menten and Manning (19), Zeckwer and Goodell (20) and others). Evans and Zeckwer (21) showed that this hyperglycemia was prevented if one adrenal was removed and the opposite splanchnic nerve cut some time previously. The hyperglycemia induced by killed bacteria in the intact rabbit was therefore considered to be due to discharge of glycogen from the liver. Thyroidectomy had practically the same effect as removing one adrenal and cutting the opposite splanchnic nerve, that is, hyperglycemia did not occur after bacterial injections in thyroidectomized rabbits with adrenals intact tested at intervals after operation varying from 7 to 20 days. In other words, injections of killed bacteria induced glycogenolysis in intact rabbits and the effect was manifested as hyperglycemia. In the present experiments insulin indirectly resulted in glycogenolysis and the effect was manifested as a counteracting of the insulin effect. In both instances, glycogenolysis was inhibited by thyroidectomy. It is not that the agent (bacteria or insulin) acts on the thyroid gland and causes any change in its function, because thyroid feeding in a thyroidectomized rabbit permitted the usual hyperglycemia after bacterial injections (Evans and Zeckwer, 21). Therefore it seemed to be a question of the thyroid hormone circulating in the body, either secreted by the thyroid gland or derived from ingested thyroid substance, affecting the sensitiveness of the sympathetic nervous system.

There is no evidence that the results of the present experiments can be applied to the problem of the resistance to insulin exhibited by certain human beings. The resistance in these rabbits was measured by the physiological compensations during an emergency of low blood sugar. Such physiological compensations can not be measured in the human being.

SUMMARY

1. Six out of a series of 40 unselected rabbits showed resistance to doses of insulin larger than those causing convulsions in any normal rabbit, while 3 showed slight resistance.

2. This tolerance varied up to 66 units, and seemed to be dependent upon a spontaneous resistance which could be increased to some extent by subjecting the rabbits to ascending non-convulsant doses.

3. Refractory rabbits injected with large doses of insulin showed a rapid fall in blood sugar, which remained low for many hours, followed by spontaneous recovery without the administration of glucose.

4. After thyroidectomy, 2 very resistant rabbits showed increased sensitiveness to insulin varying from 10 to 20 fold, while 4 normal control rabbits showed an increased sensitiveness after thyroidectomy of 2.5 to 4.6 fold. However, the resistant rabbits after thyroidectomy never convulsed with the minute doses of insulin to which normal rabbits respond after thyroidec-

tomy. That is, a considerable residual resistance to insulin remains after thyroidectomy in the very refractory rabbits.

5. The thyroids of resistant rabbits showed no gross or microscopic deviation from normal.

6. The relation of refractoriness to activity of the sympathetic nervous system is discussed.

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THE INFLUENCE OF NERVOUS STIMULATION ON ABSORPTION FROM THE INTESTINE

A Contribution to the Humoral Theory of Nervous Action[1]

ERNST GELLHORN and DAVID NORTHUP

From the Department of Physiology, College of Medicine, University of Illinois, Chicago

Received for publication July 3, 1933

In two previous papers (Gellhorn and Northup, 1933a, b) it was shown that in a frog preparation in which the blood vessels supplying the gut, and the gut itself, were perfused separately, different hormones showed specific effects on the permeability of the gut membrane. It was of particular interest that acetylcholin and adrenalin showed antagonistic effects in regard to the permeability of the gut. In high concentrations (adrenalin 1:500,000 to 1:1,000,000 and acetylcholin 1:50,000 to 1:500,000) adrenalin increased and acetylcholin decreased the permeability of the gut to glucose, whereas in 10 to 50 times lower concentrations the effects were reversed. Since the amount of perfusion fluid was kept constant, the changes are independent of the vascular effects of the hormones.

The regularity with which these results were obtained and the great sensitivity of this preparation to adrenalin and acetylcholin made it probable that this method would be suitable to test in another way the theory which was first advanced by O. Loewi that the stimulation of autonomic nerves leads to the liberation of definite chemical substances.

That the stimulation of the parasympathetic leads to the formation or liberation of acetylcholin has been shown by Loewi and his co-workers in regard to the heart (1921–26), by Engelhardt (1930) in regard to the oculomotor nerve and more recently by v. Beznak (1932) and Babkin, Gibbs and co-workers (1932) in the salivary gland. Feldberg (1933) showed the appearance of an acetylcholin like substance after stimulation of the lingual nerve in the veins of the tongue. There is still some disagreement as to the liberation of acetylcholin in the heart after vagus stimulation. In numerous papers Asher and co-workers (compare Asher in Gellhorn's *Textbook of general physiology*, 1931, p. 621, and Asher and Scheinfinkel, 1932) tried to show that the effects of vagus stimulation in the heart lead to the Loewi effect only in case the heart is in a hypodynamic state; but it may be

[1] Aided by a grant from the Ella Sachs Plotz Foundation.

mentioned that Kahn (1926) confirmed Loewi's result in the heart with a different method.

There is considerable evidence of the liberation of adrenalin or an adrenalin-like substance after the stimulation of the sympathetic. In experiments on the frog's heart Loewi gave the first proofs of a humoral theory of nervous stimulation of the sympathetic. That this principle holds for the stimulation of the sympathetic in the gut was shown by Finkleman (1930) and more evidence of the appearance of an adrenalin-like substance after sympathetic stimulation of smooth muscle was given recently by Cannon and co-workers (Newton, Zwemer and Cannon, 1931; Cannon and Bacq, 1931; Rosenblueth and Phillips, 1932; Rosenblueth and Cannon, 1932). Whether or not the substance liberated is adrenalin or a substance of similar characteristics (Cannon's sympathin) is still undecided. Finally the work of Lehmann (1932) may be mentioned in which it was found that after sympathetic stimulation the perfusion fluid of a Läwen-Trendelenburg preparation (perfusion of the hind legs in the frog) contained an adrenalin-like substance according to a test experiment with a frog's heart.

In most of the methods by which the humoral theory of nervous action was studied a sample of the fluid or blood which supposedly contained the substance liberated under the influence of vagus or sympathetic stimulation was withdrawn and studied in another (test) preparation. This procedure involves a considerable loss since it must be assumed that only a fraction of the substance formed under the influence of nervous stimulation will diffuse into the blood or perfusion fluid. It seemed therefore desirable to study this question with a preparation used in our previous work in which characteristic changes in sugar absorption were found after acetylcholin and adrenalin administration. If nervous stimulation would lead to a liberation of acetylcholin and adrenalin respectively the same changes in the permeability of the gut should be observed as were obtained after the addition of these drugs in proper concentrations.

METHOD. The preparation used was a frog, set up as described in an earlier paper (Gellhorn and Northup, 1933a). The spleen was ligated to exclude it from the gut circulation, as traces of blood sometimes appeared in the perfusate when it had gone through the spleen. Ringer's solution was used for the perfusion of the capillaries. It was found that by reducing the $CaCl_2$ concentration from 0.01 per cent to 0.005 per cent a tendency toward a declining permeability could in most cases be overcome, without producing any increasing permeability. The permeability at the start of the experiment was usually higher than when Ringer's with 0.01 per cent $CaCl_2$ was used, but it remained, in control experiments, at a constant level.

For stimulation of the vagus nerve, an electrode was plunged into the

medulla, which, along with the cervical cord, was kept intact in this type of experiment. A second electrode was placed in the muscles near the vertebral column. The stimulation was intermittent during the "stimulation periods," and was always at least strong enough to produce a marked slowing of the heart. This type of stimulation produced no noticeable effect on the perfusion rate.

For stimulation of the splanchnics several procedures were tried. One electrode in the thoracic cord, with the indifferent one either fine and near the different, or large and applied to the dorsal surface of the animal, proved to be unsatisfactory. It was finally found that a pair of electrodes applied to the splanchnic nerves where they accompany the anterior mesenteric artery gave satisfactory results. The stimulation was, as with the vagus, intermittent. It produced a marked constriction of the vascular bed, necessitating a rise in perfusion pressure to hold the rate of perfusion constant. And due to excitation of the sympathetic chain, probably due to spread of current (the splanchnics are very close to the sympathetic chain at the point stimulated), a marked acceleration of the heart was noted during stimulation. When strong stimuli were used this acceleration sometimes lasted long beyond the cessation of stimulation, possibly indicating that some irritative process had been set up by the electric current. In all experiments reported here the electrodes were in place only during actual stimulation. As in our previous work the gut was perfused with 3.15 per cent glucose and the blood vessels with well oxygenated sugar-free Ringer's solution. This concentration was used because it is isotonic and because with it a sufficient quantity of glucose is absorbed in a single circulation to allow of adequate analytical accuracy.

Twenty-four experiments were performed in this manner; twelve concerned vagus and an equal number sympathetic stimulation. The most surprising fact was that in *all* experiments the rate of absorption was altered. Since the rate of perfusion was kept constant and, in control experiments, without stimulation of these nerves the sugar concentration remained practically unchanged in two hours' observation, *the experiments indicate that nervous stimulation alters the permeability of the gut.* This fact is in good agreement with our previous studies on the influence of nervous stimulation on the K-contracture (1931) which made it probable that stimulation of somatic nerves increases permeability. It may therefore be said that the *stimulation of both somatic and autonomic nerves alters cellular permeability.*

As to the character of the changes in permeability, it is necessary to discuss the experiments with stimulation of the vagus and of the sympathetic nerve separately and in detail. Table 1 gives a brief survey of the results obtained. It is apparent that after the stimulation of either nerve the permeability of the gut may be either decreased or increased. It

might appear that the effect is purely random and due to spontaneous fluctuations independent of stimulation; but this is seen not to be true because in control experiments, as illustrated in table 2, there are no changes of this order of magnitude. We have some doubts in regard to the significance of the fact that in the majority of our experiments the permeability was decreased. It seems probable that what result occurs depends on the

TABLE 1

Influence of vagus and sympathetic stimulation on the permeability of the gut to glucose

1. Vagus stimulation
10 experiments show decrease in permeability*
2 experiments show increase in permeability
2. Sympathetic stimulation
8 experiments show decrease in permeability
4 experiments show increase in permeability†

* In 6 experiments irreversible decrease.
† In 3 experiments irreversible increase.

TABLE 2

PERFUSION SAMPLE NUMBER	GLUCOSE IN MGM.%					
1	2.6*	1.7*	3.7*	5.0*	12.6†	11.3†
2	2.2	1.7	3.6	5.3	12.3	11.6
3	2.4	1.6	3.6	5.0	13.0	10.2
4	2.1	1.6	3.4	4.8	13.0	9.3
5	2.5	1.5	3.3	5.0	12.3	10.8
6	2.0	1.6	3.1	5.0	13.0	9.9
7	2.1	1.7	3.6	4.8	14.2	10.8
8	2.3		3.4	5.0	14.2	11.2
9	1.8				12.7	9.4
10					12.8	9.7

* Ringer's with 0.01 per cent $CaCl_2$.
† Ringer's with 0.005 per cent $CaCl_2$.

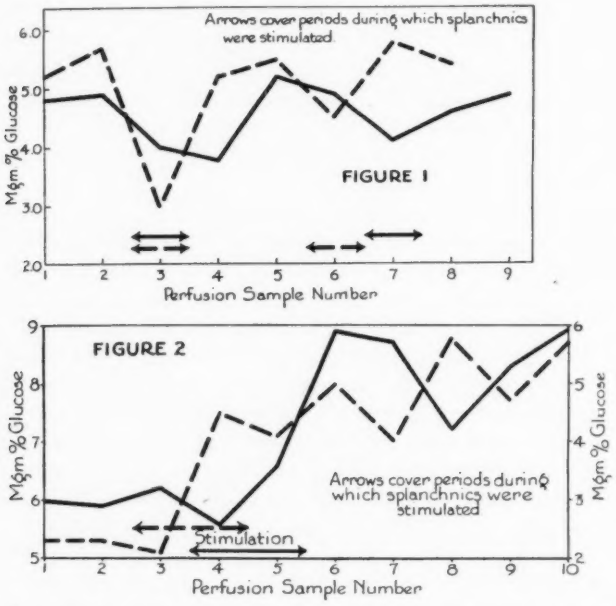
intensity of the nervous stimulation. This in turn means in the light of the theory discussed above that the alteration in the membrane characteristics depends on the amount of substance liberated under the influence of nervous stimulation. Such an assumption easily explains our findings since it has been shown in previous papers (1933a, b) that acetylcholin as well as adrenalin either increases or decreases the permeability of the gut to glucose, depending solely upon concentration. As was described in our earlier work the significant difference in the effect of

these substances on the gut was the fact that acetylcholin increased glucose absorption in low concentrations, as did adrenalin in high ones and that the adrenalin effect was much stronger. Furthermore, a decrease in glucose absorption at a constant perfusion rate was observed with adrenalin in very low concentrations and with acetylcholin in high ones. This makes it probable that if Loewi's theory is correct and applicable to our experimental findings, an increase in permeability ought to be characteristic of a strong stimulation of the sympathetic and a decrease of a strong stimulation of the vagus nerve. Unfortunately a classification can not be made on the basis of the strength of the stimulus which varied from 8 to 0 cm. distance between primary and secondary coil of the induction apparatus, since the effects obtained do not show a clear dependence of the type of reaction on the intensity of the stimulus. This is probably due to the fact that the excitability of the nerves varies greatly in different preparations. Therefore no direct comparison of the intensity of stimulation is valid for various preparations.

Assuming the validity of the humoral theory of nervous action and taking into account that the preparation used in these experiments consists chiefly of a part of the gut and the blood vessels and nerves supplying these tissues, the effect of nervous stimulation on sugar absorption must be due to the liberation of chemical substances which alter the permeability of the gut if present in minute concentrations. Finkleman showed that adrenalin or an adrenalin-like substance is produced after stimulation of the sympathetic in the gut and Le Heux (1919–1921) proved the presence of cholin and acetylcholin in the gut wall. These observations together with our own findings that the alterations in the permeability of the gut after nervous stimulation are similar to those found after administration of adrenalin and acetylcholin respectively make it highly probable that nervous stimulation alters the glucose absorption by means of these chemical substances. There are further indications in this work that this is correct. In our previous experiments it was frequently found that after the administration of various hormones a prolonged after-effect was noted; the effect was not immediately reversible upon exchanging the hormone-containing solution for Ringer's but continued more or less unchanged for several periods. It is now interesting to note that such irreversible effects were also observed after nervous stimulation and were quite different for vagus and for sympathetic stimulation. *Whenever an irreversible effect in regard to glucose absorption occurred, it was always a decrease after vagus stimulation and regularly an increase after sympathetic stimulation.* This is exactly the result to be expected if relatively large concentrations of acetylcholin and adrenalin are liberated after vagus and sympathetic stimulation respectively.

Figure 1 is an example of the reversible decrease in glucose absorption

after splanchnic stimulation. During the periods of stimulation vasoconstriction occurred, but was compensated at once by raising the perfusion pressure, thus keeping the perfusion rate constant. In the experiment of figure 2 the intermittent stimulation of the sympathetic (alternately 30 seconds' stimulation and 30 seconds' rest) was carried out over two periods (20 minutes) and produced a very prolonged increased permeability to glucose. It is noteworthy that the rate of the heart beat remained increased after the period of stimulation was over. This seems



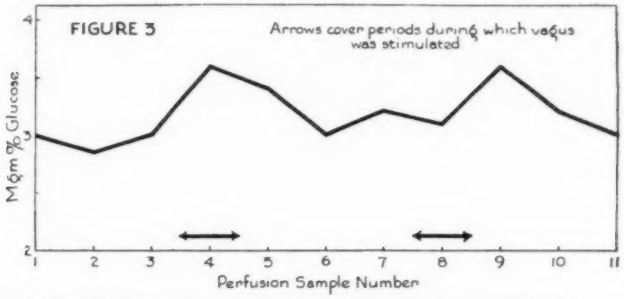
Figs. 1 and 2. The influence of stimulation of the splanchnics on the absorption of glucose from the gut under conditions under which the perfusion rate of the blood vessels of the gut remains unaltered. Dash line indicates a duplicate experiment.

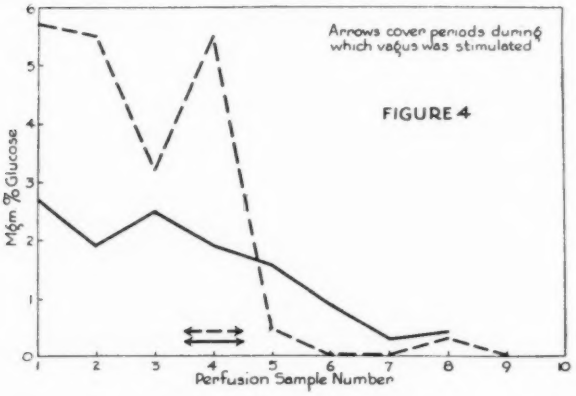
to indicate that the strong stimulation has caused some irreversible alteration of the nerve which led to a prolonged liberation of adrenalin.

Figure 3 is a typical example of the reversible increase in glucose absorption after vagus stimulation. Similarly as in the experiments with administration of acetylcholin, no effect on the blood vessels was observed. As an example of the prolonged decrease in permeability of the gut after vagus stimulation figure 4 is reproduced. The effect became apparent in the period following the stimulation period and in the subsequent ones. This delayed effect was frequently observed in our previous work when the

hormones were added to the circulation fluid. The interrupted curve representing the permeability to glucose is particularly impressive since the glucose concentration in the blood vessels dropped from about 5 mgm. per cent to almost 0 on account of the vagus stimulation.

The nature of the changes occurring in the gut under these conditions will be discussed in a subsequent paper.





Figs. 3 and 4. The influence of vagus stimulation under similar conditions.

SUMMARY

In a frog preparation in which the gut is perfused with isotonic glucose and the blood vessels supplying the gut with Ringer's solution and in which the perfusion rate is kept constant the absorption of glucose is regularly altered by stimulation of the autonomic nerves. The results are similar to those obtained by administration of acetylcholin and adrenalin respectively. They are in favor of the humoral theory of the action of autonomic nerves. Furthermore they prove that autonomic nerves alter cellular permeability.

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PHYSIOLOGICAL EFFECTS OF HIGH FREQUENCY CURRENT

IV. An Estimate of the Energy Requirement of Pulmonary Hyperventilation[1]

E. S. NASSET and S. B. PETERS

From the Department of Vital Economics, University of Rochester, Rochester, N. Y.

Received for publication July 5, 1933

The problem of the energy requirement of the respiratory muscles is not new. Finkler and Oertmann (1877) determined the respiratory metabolism of rabbits under basal conditions and during apnea, produced and maintained by artificial respiration. Since they detected no appreciable difference in oxygen consumption between basal and experimental conditions, their conclusion was that respiratory movements exerted no influence upon the total respiratory metabolism. In experiments upon himself, Speck (1892) showed that a voluntary increase of the respiratory volume resulted in a 3 per cent increase of oxygen consumption, per liter of extra ventilation per minute. Bornstein and von Gartzen (1905) demonstrated a 60 per cent increase in total oxygen consumption in a human subject during voluntary forced breathing. Liljestrand (1918) reported experiments upon himself and two other human subjects. The changes in ventilation were effected by rebreathing and by voluntary forced respiration. Calculated in terms of the percentage increase in total oxygen consumed, per liter of extra ventilation, his rebreathing experiments give average values of 1.2 per cent; the forced respiration experiments, 4.4 per cent. He ascribed this difference to the fact that in the latter type of experiment, expiration ceases to be a passive process and becomes an active one.

It is probable that the earlier investigators, who compared the oxygen consumption of small animals under conditions of normal spontaneous breathing and of apnea, failed to find any difference because the magnitude of the change was less than the error of the method employed in the determination of respiratory metabolism.

The suggestion was made, in another paper of this series (Nasset, 1932), that the increased oxygen consumption noted in acute hyperthermia in anesthetized dogs was not greater than might be expected from the opera-

[1] We wish to acknowledge the financial aid of The Rockefeller Foundation for Medical Research in the work described in papers II, III and IV of this series.

tion of two main factors, namely, high body temperature as such and the extreme activity of the respiratory muscles. The experiments herein described represent an attempt to determine, separately, these two factors in the same animal.

METHODS. Dogs served as the experimental animals. Sodium amytal was the anesthetic except in two cases in which chloretone was used. The expired air was collected in a gasometer and analyzed in a Haldane type analyzer. Three types of experimental procedure were used: 1, rebreathing; 2, simultaneous warming of the carotid and cooling of the jugular blood, and 3, crossed-circulation.

In the rebreathing experiments the dead space was increased by mechanical means. We were unable by this method to induce more than a fourfold increase in pulmonary ventilation. The rate of oxygen consumption never exceeded the basal rate by more than 8 per cent. It is significant for our purpose that oxygen consumption was essentially unaltered in those animals whose ventilation was less than trebled.

From a consideration of some of the work of Richet (1898) and of Heymans (1921) it appeared that heat polypnea might be largely of central origin. In the attempt to heat the central nervous system (CNS) without raising the temperature of the trunk, the vertebral vessels were ligated and the carotids and jugulars placed in thin-walled copper jackets through which water at any desired temperature could be circulated. By thus warming the carotid and cooling the jugular blood it was possible to attain a maximum difference of 1.5°C. between oral and rectal temperature. This difference was apparently insufficient to lead to any manifest alteration of the respiration.

In order to effect a greater difference between the temperatures of the brain and trunk we resorted to crossed-circulation experiments. The donor animal ("A") was usually larger than the recipient ("B"). The common carotid arteries and external jugular veins of both animals were dissected free over the whole length of the neck. By means of Payr cannulae, carotid to carotid and jugular to jugular anastomoses were made (fig. 1). This provided intima to intima junctions which obviated the use of anticoagulants. The vertebral vessels in "B" were ligated at their point of entrance to the spinal column. A chain clamp was placed about the neck at about the fourth cervical vertebra to functionally isolate the head from the trunk, excepting the vagi and spinal cord connections which were left intact. The dog table tilted up on the recipient's side so that gravity would assist the venous return from the perfused head of "B." It was found advisable also to maintain "B" somewhat higher (8–10 cm.) at the tail than at the head to prevent a return of blood from the head via the spinal veins. Bitemporal electrodes on "B" and body electrodes on "A" served to raise the temperature of the CNS of "B." With the passage of

high frequency current the body temperature of "A" could be elevated to any desired height, perfusing the CNS of "B" with blood above the temperature of his own trunk. Conversely, it was possible to place electrodes on the trunk of "B" and so to raise his rectal temperature while maintaining the temperature of the CNS normal. These combinations were possible because there was very little mixing of the bloods of the two animals. Injections of epinephrine into one dog sufficient to cause a large rise in

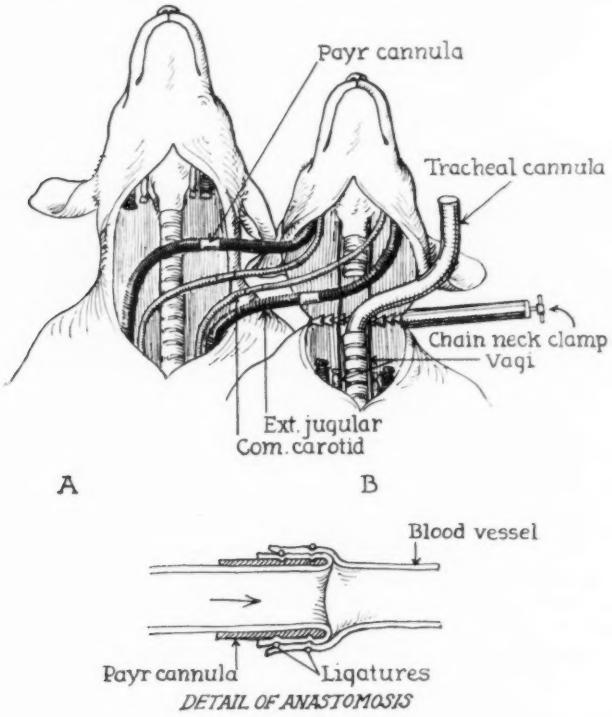

Fig. 1

blood pressure only occasionally showed a slight effect in the other. Furthermore, hematocrit, sugar and non-protein nitrogen determinations showed differences between the two animals which were maintained throughout the experiment (4–8 hours).

Results. As stated above, the rebreathing type of experiment failed to yield the magnitude of response desired. However, the observation that the ventilation of the amytalized dog could double or treble without appreciably affecting the oxygen consumption was very useful. Mindful

of this fact, we selected from our whole series those experiments in which the ventilation was less than trebled but in which, nevertheless, there was a considerable rise of rectal temperature (> 1°C.). The increase in oxygen consumption in these cases was taken as a measure of the influence of heat as such upon the respiratory metabolism. Using the formula suggested by Bazett (1927), we have computed the Q_{10} for oxygen consumption in these experiments (table 1).

Illustrative examples of thirteen successful crossed-circulation experiments are given in table 2. The data recorded here were all obtained from dog "B," i.e., the dog whose head was perfused. It may be noted that

TABLE 1

Q_{10} for O_2 consumption in hyperthermia

EXPERIMENT NUMBER	RANGE OF RECTAL TEMPERATURE	Q_{10}	ANESTHETIC	PYRETIC AGENT	REMARKS
34	36.7–39.6	1.04	Amytal	H.F.C.	Single dogs
35	37.1–39.5	2.27	Amytal	H.F.C.	
35	39.1–40.3	4.23	Amytal	H.F.C.	
36	39.3–40.2	1.97	Amytal	H.F.C.	
37	37.6–39.6	1.43	Amytal	H.F.C.	
38	39.7–40.9	1.85	Amytal	Infection	
42	37.5–40.0	1.24	Amytal	Hot H_2O to carotid	
45	35.1–37.6	4.46	Chloretone		
45	37.6–39.1	1.79	Chloretone		
46	37.1–39.1	3.30	Chloretone		
102	38.7–42.4	2.49	Amytal	H.F.C.	"B" dog in X-C experiments
104	37.5–40.9	1.63	Amytal	H.F.C.	
106	37.5–41.0	2.42	Amytal	H.F.C.	
Average.....		2.32			

bringing the oral temperature to 41–42°C. had little if any influence upon respiration. Likewise, heating the trunk alone to the same degree had little effect, although in most cases the effect was greater than that obtained by heating the head alone. If first the head were heated to fever temperature and then the trunk brought to approximately the same temperature, the animal gave the respiratory response typical of a single intact anesthetized animal subjected to the same elevation of body temperature. Whether the head or the trunk be heated first apparently makes no difference in the end result.

It was obviously impossible under these conditions to dissociate hyperventilation from a generalized elevation of body temperature. Failing

to make a direct determination of the energy requirement of the respiratory movements, we employed the following indirect method to arrive at tentative values. Using $Q_{10} = 2.32$ for all hyperthermia experiments, the oxygen consumption due to a rise in body temperature was calculated and added to the basal value. This sum subtracted from the experimentally

TABLE 2

Representative crossed-circulation experiments. Data from dog "B"

EXPERIMENT NUMBER	TEMPERATURE		VENTILATION PER MINUTE	O_2 CONSUMPTION PER MINUTE	RESPIRATION PER MINUTE	REMARKS
	Rectal	Oral	*liters*	*cc.*		
94	38.9	37.8	10.45	127	40	1st sample after crossing
	39.1	39.1	9.13	128	39	Bitemporal electrodes on "B"
	39.2	40.0	12.93	139	51	Abdominal electrodes on "A"
	39.2	41.7	16.77	153	65	
104	37.2	35.9	4.59	86	18	Vertebrals open. Clamp on. No X-C
	37.5	36.8	4.27	85	19	Vertebrals tied. X-C established
	40.9	37.0	4.91	100	24	Abdominal electrodes on "B"
	42.3	35.9	5.69	99	38	Abdominal electrodes on "B"
106	37.4	36.3	4.05	90	14	Vertebrals open. Clamp on. No X-C
	37.5	36.5	3.77	81	15	Vertebrals tied. X-C established
	41.0	37.8	8.51	110	49	Thoracic electrodes on "B"
	41.5	40.1	16.17	118	137	Thoracic + bitemporal electrodes on "B"
	42.4	40.9	33.41	134	180	Thoracic + bitemporal electrodes on "B"
124	35.7	35.3	3.78	112	8	Vertebrals open. Clamp on. No X-C
	37.6	40.4	5.02	124	8	Vertebrals tied. X-C. HF
	38.5	41.3	5.29	126	11	Abdominal electrodes on "A." Bitemporal on "B"
	40.5	41.0	10.06	163	25	Abdominal electrodes on "A and B." Bitemporal on "B"
	42.2	41.8	25.22	192	66	Abdominal electrodes on "A and B." Bitemporal on "B"
	42.4	41.7	33.64	188	114	Abdominal electrodes on "A and B." Bitemporal on "B"

"A" = donor, "B" = recipient. X-C = Crossed circulation.

observed total gave a remainder which was taken to represent the oxygen requirement of the respiratory muscles ("cost of respiration"). This value was expressed also as the percentage increase in oxygen consumption per minute per liter of excess ventilation per minute (table 3). This amounts to an average of 0.8 per cent for all experiments.

DISCUSSION. We are aware that the amytalized animal is not a suit-

able subject for respiration studies and hence we do not present these experiments as a contribution to the physiology of normal respiration. They represent merely an attempt to dissociate, under specific conditions, the high rate of oxygen consumption observed in hyperthermia experiments into two main factors, namely, "cost of respiration" and the direct effect of heat as such. In order to make the results applicable to our earlier experiments it was necessary to maintain as far as possible the same experimental conditions.

TABLE 3

Calculation of the energy requirement of pulmonary ventilation

EXPERIMENT NUMBER	OXYGEN CONSUMED, PER MIN.			VENTILATION COEFFICIENT*	PER CENT INCREASE IN O_2/MIN./L. INC./MIN. IN VENTILATION	REMARKS
	Observed total	Calc. due to heat $Q_{10} = 2.32$	Diff. "Cost of resp."			
	cc.	cc.	cc.			
34	81					Amytal
	139	116	23	6.5	1.13	High frequency
	172	127	44	9.0	1.37	Current (HF)
	174	127	47	8.8	1.48	Heating
35	124					Amytal
	202	189	13	5.1	0.40	HF
	246	202	47	7.4	0.89	
102	116					Amytal, X-circ.
	174	164	10	6.3	0.40	HF
	266	160	84	12.7	1.54	
106	81					Amytal. X-circ.
	118	113	5	4.3	0.33	HF
	136	122	12	8.9	0.33	

* Number of times as great as basal ventilation.

It is evident that the polypnea encountered in amytalized dogs heated with high frequency current is not primarily of central origin. If this were the case a marked response should have been elicited on heating the CNS above normal temperature. Furthermore, the polypnea is not wholly dependent upon the temperature of the trunk, or in effect the periphery. Although the temperature of the trunk appears to exert a greater influence upon the initiation of the respiratory response to heat, it cannot call forth the maximum response unless the CNS be at a high temperature also. The factors governing this coöperation are still obscure; it may be that as the CNS becomes warmer it becomes more reactive to the afferent volley of impulses arriving from the temperature sense receptors.

As judged by the oral and rectal temperatures, and the oxygen consumption, there is no indication that raising the temperature of the CNS causes an increase in heat loss or a decrease in heat production by the body. This dissimilarity to the results reported by Barbour (1912) and by Hashimoto (1915) for the rabbit may be due to the use of anesthesia, a different means and degree of heating and the like.

Admittedly the Q_{10} varies widely for different experiments and for different temperature ranges in the same experiment. We have no satisfactory explanation for this. Certainly one important consideration is the method of determining body temperature. It is obvious that the rectal temperature alone cannot give a representative average of the temperature of all actively respiring tissue at different stages of fever induction. Moreover, when the rate of oxygen consumption is rapidly changing, its direct determination over a short period (3–10 min.) may not be an accurate index of the extent of oxidation actually occurring within that period. The observed variability might be explained on the basis of these two considerations. The Q_{10} is presented merely as a necessary step in the indirect solution of a problem, the direct solution of which we failed to achieve.

It may be noted (table 3) that, in general, as the respiratory activity is intensified the percentage increase in oxygen consumption, per liter increase in ventilation, becomes much greater. This is in agreement with the well known fact that muscular efficiency decreases as the rate of work exceeds a certain optimum. The computed oxygen requirement of the respiratory muscles in our experiments is about two-thirds as great as the value obtained by Liljestrand (1918), using the rebreathing technic in direct determinations on man. This may be an indication that the respiratory muscles of the dog are more efficient than those of man at pulmonary overventilation. This idea also receives support from the observed fact that the dog may double or treble his ventilation without appreciably increasing the total oxygen consumption. It is well to emphasize again the fact that these experiments were done with amytalized dogs and therefore the data cannot be indiscriminately applied to the interpretation of normal physiological processes.

These experiments further confirm our opinion that high frequency electric current (10^6 cycles per sec.) does not exert any specific influence upon the oxygen consumption of the amytalized dog. The fact that the calculated "cost of respiration" is considerably lower than in man leaves scant room for the demonstration of any such specific effect.

SUMMARY

1. A crossed-circulation technic is described.
2. The amytalized dog may be induced, by rebreathing, to double or

treble his pulmonary ventilation without appreciably increasing total oxygen consumption.

3. The polypnea of experimentally induced hyperthermia in amytalized dogs is neither wholly of central nor of peripheral origin, and the typical response is obtained only by the simultaneous heating of both CNS and periphery.

4. The energy requirement of pulmonary hyperventilation, or the "cost of respiration," (percentage increase in oxygen per minute per liter increase in ventilation per minute) probably is less for the dog than for man.

We are happy to express our thanks to Dr. Rene Gayet of Paris for demonstrating some of the details of his crossed-circulation technic, a modification of which has been used in this work.

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METABOLIC STUDIES IN PARTIALLY HYPOPHYSECTOMIZED DOGS

RAY G. DAGGS AND A. G. EATON

From the Department of Vital Economics, University of Rochester, Rochester, N. Y.

Received for publication July 5, 1933

Many of the older studies led to the conclusion that the hypophysis was essential to life, but later work, notably that of Sweet and Allen (1913), Brown (1922–23), Camus and Roussy (1922) and Sachs and MacDonald (1925), has clearly demonstrated that the gland may be removed without endangering life and that many of the symptoms attributed to hypophyseal removal were due to injuries to the hypothalamic region of the base of the brain.

I. THE BASAL METABOLIC RATE AND THE SPECIFIC DYNAMIC ACTION OF MEAT. Several clinical investigators have shown the basal metabolic rate to be lowered in cases of hypopituitary conditions and increased in hyperpituitary conditions. Plaut and her co-workers (Plaut, 1923; Liebeschutz-Plaut, 1925; Liebeschutz-Plaut and Schadow, 1925; Kestner, Liebeschutz-Plaut and Schadow, 1926) have reported many times a lowered specific dynamic action of meat in patients suffering from diseases of the pituitary. She believes this to be of such constancy as to be of great diagnostic value. Liebesny (1924) also found a decreased specific dynamic action of meat in hypopituitary cases. When anterior lobe preparations were administered the specific dynamic action increased. Knipping (1923) produced operative injury to the hypophysis in dogs and obtained a lowered specific dynamic action which returned to normal upon the administration of anterior lobe substance. Working with hypophysectomized rats Foster and Smith (1926) found no specific dynamic action following the administration of glycine. The combined extracts of both lobes of the hypophysis restored it. They also demonstrated a lowered basal metabolic rate due to the removal of the anterior lobe. Artundo (1931a) studied the basal metabolism of eight hypophysectomized dogs. It was normal in one dog, nearly normal in two dogs and reduced in the other five from 0 to a minus 32 per cent with an average of minus 15 per cent. Artundo quotes Grafe and Grunthal as having shown a lowered basal in eight of thirteen dogs with lesions of the tuber cinereum. Gaebler (1929) was one of the first to report no significant change in the specific dynamic action of a dog after hypophysectomy. Artundo (1931b) found the specific dynamic action

of six hypophysectomized dogs to be the same as in normals when the extra heat was calculated as calories increase over basal but greater when calculated on the percentage basis because of the lowered basal. From the clinical standpoint Johnston (1932), in refutation of the earlier work, reported normal specific dynamic action in six cases of definite pituitary abnormalities.

Herzfeld (1930) showed the specific dynamic action to be intensified in normal human subjects after several days' injection of anterior lobe extract. Nitzescu and Gavrila (1929) reported the basal metabolism to be increased after injections of pitressin and no particular effect following the administration of oxytocin. Working with guinea pigs Verzar and Wahl (1931) found the anterior lobe to be responsible for the increase in metabolic rate following its administration. The rise increased continually for a long time when injections were given daily. They attribute this increase to the discharge of colloidal substance of the thyroid gland since this substance disappears under the conditions of the experiment and after the extirpation of the thyroid gland, injections of the hypophyseal hormone are no longer effective in raising the metabolism, but cause a considerable fall in metabolism. Gaebler (1933) administered solutions of the globulin fraction of the alkali extracts of the anterior lobe of beef pituitary intravenously or subcutaneously to dogs and obtained large increases in the heat production. Lee and Gagnon (1930) after feeding desiccated anterior lobe pituitary substance to rats for 62 days were unable to demonstrate any effect upon the basal respiratory metabolism or the respiratory quotient.

In summary we might say that the bulk of the recent work tends to authenticate the statement that the basal metabolism is lowered following hypophysectomy due to the absence of a substance from the anterior lobe and that the specific dynamic action of meat is unaltered if calculated as caloric increase over basal.

Experimental. Two large male dogs were hypophysectomized using the McLean technic (1928). The operations were performed by John J. Jares and Michael J. Lepore under the supervision of Prof. William P. VanWagenen. One year later two students, Mr. George Dacks and Mr. Elbert Dalton obtained a positive Cowley test on each dog indicating the absence of the hypophysis. Their data are shown in table 1. If the blood from a post-absorptive hypophysectomized dog injected intraperitoneally into a rabbit causes a marked hypoglycemia, the test is considered positive.

Eighteen months later we obtained a negative Cowley test in both dogs. This indicated one or more of three things: that the test is unreliable, that there had been some compensating mechanism set up, or that regeneration of the hypophyseal tissue had occurred. We prefer the last explanation in light of the histological findings at autopsy.

The basal metabolism and also the specific dynamic action of beef heart were determined on these dogs using the Tissot method. The dogs were trained to lie still with a face mask and breathe into the spirometer. After two satisfactory basal periods on the dogs in the post-absorptive state they were given 1000 grams of lean beef heart, ground fine and heated to body temperature. They consumed the entire amount of meat in about five minutes. The expired air was collected for 10 minute periods every hour for 5 hours following the ingestion of the meat. The air was analysed in duplicate on the Henderson-Haldane gas analyzer. Table 2 shows the average of five to ten basal determinations for each dog. All three dogs were of approximately the same weight.

TABLE 1

	BLOOD SUGAR (Benedict modication of Folin-Wu method)			
	Normal	1 hour	2 hours	Hypoglycemic action
	mgm. per cent			*per cent*
Dog I	111	106	54	−53
Dog II	97	75	47	−51
Control	90	84	78	−13

TABLE 2

	BASAL METABOLISM Calories per square meter per hour ($0.112 \times W^{2/3}$)	
	Calories	Change in basal
		per cent
Control*	32.49	
Dog I	25.14	−22.62
Dog II	30.74	−5.38

* A female of the same size as the experimental animals and which had been accustomed to cage life for approximately the same length of time.

The results show a significant lowering in the case of dog I but not in the case of dog II. There is some histological evidence for tuber cinereal injury in dog I which showed the lower basal metabolic rate. There was a cyst-like structure attached to the tuber cinereal region. This is interesting in the light of the findings of Grafe and Grunthal, who demonstrated lowered basal metabolism in dogs with lesions of the tuber cinereum. Dog II showed no histological evidence of injury to the tuber cinereum. There was less anterior lobe surviving in dog I and it was rather loosely arranged indicating the probability of its being regenerated tissue. Dog II had a rather large amount of normal appearing anterior lobe tissue remaining.

In view of the work of Foster and Smith, Verzar and Wahl, Herzfeld, Gaebler and others, it seems probable that the lessened decrease in the basal metabolism of dog II is due to the presence of a larger amount of anterior lobe tissue.

Table 3 shows the average of five experiments on the specific dynamic action on each dog.

The results show no indication of a lowered specific dynamic action due to hypophyseal disturbance, but rather, if anything, a slight increase. Foster and Smith believe that the substances of both lobes are necessary

TABLE 3

Specific dynamic action of 1000 grams of beef heart. Average caloric increase over basal for a five-hour period

DOG	EXPERIMENT	BASAL CALORIES PER HOUR	1ST HOUR		2ND HOUR		3RD HOUR		4TH HOUR		5TH HOUR		AVERAGE CALORIC INCREASE OVER BASAL
			Calories per hour	Caloric increase over basal	Calories per hour	Caloric increase over basal	Calories per hour	Caloric increase over basal	Calories per hour	Caloric increase over basal	Calories per hour	Caloric increase over basal	
I	1	26.86	39.24	12.38	39.99	13.13	42.89	16.03	42.55	15.69	42.13	15.27	14.50
I	2	27.51	39.16	11.65	41.55	14.04	44.68	17.17	45.38	17.87	44.28	16.77	15.50
I	3	25.68	36.83	11.15	43.07	17.39	44.40	18.72	41.97	16.29	34.87	9.09	14.53
I	4	27.55	39.06	11.51	42.83	15.28	45.16	17.61	42.34	14.79	43.91	16.36	15.11
I	5	26.79	40.00	13.22	44.89	18.11	45.30	18.52	44.74	17.96	44.45	17.67	17.10
II	1	29.27	40.67	11.40	42.90	13.63	46.31	17.04	47.15	17.88	46.52	17.25	15.44
II	2	31.89	44.17	12.28	45.99	14.10	46.67	14.78	47.32	15.43	47.66	15.77	14.47
II	3	33.46	48.77	15.31	50.10	16.64	51.59	18.13	52.98	19.52	50.98	17.42	17.42
II	4	33.22	44.02	10.80	47.37	14.15	51.34	18.12	51.61	18.39	51.70	18.48	15.99
II	5	32.01	45.94	13.93	51.00	18.99	50.35	18.34	49.74	17.73	45.97	13.96	16.59
Normal	1	35.18	47.09	11.91	47.70	12.52			45.22	10.04	44.98	9.80	11.07
	2	35.18	45.94	10.76			48.34	13.16	47.65	12.47	44.23	9.05	11.36
													13.25 (Lusk)

for normal specific dynamic action. The histological sections indicate that both lobes were functionally present in our dogs.

II. THE INSULIN TOLERANCE AND THE GLUCOSE TOLERANCE. There is plenty of evidence to show a relationship of the pituitary body to carbohydrate metabolism the most striking of which comes from Houssay's laboratory, lately confirmed by Regan and Barnes (1933). Houssay and Biasotti (1931) have summarized this relationship as follows:

1. Slight glycosuria after hypophysectomy.
2. Increased carbohydrate tolerance and decreased insulin tolerance after hypophysectomy.

3. Antagonism of insulin and the pituitary and the protection of post-pituitary extracts.

4. Non-appearance of typical and severe diabetes with marked hyperglycemia in the toad after hypophysectomy or anterior lobe extirpation and pancreatectomy. Then the appearance of diabetes upon the implantation of the anterior lobe.

5. The intolerance of hypophysectomized dogs to phlorhizin.

These workers (1930) found that removal of the hypophysis in dogs did not entirely prevent diabetes but lessened its intensity. Several years ago Cushing (1912) demonstrated an increased sugar tolerance and a definite hypoglycemia in cases of hypopituitarism. Cushing and his co-workers (1911) believed this increased sugar tolerance to be due to the loss of the posterior lobe. Eidelsberg (1932) has recently confirmed these findings and also states that in many cases of hyperpituitarism the sugar tolerance is decreased. Burn (1923) and others have demonstrated the antagonism between pituitrin and insulin. Geiling (1927) showed that the injection of insulin into hypophysectomized dogs resulted in an increased effect over normal and that the dogs were more subject to convulsions. He believed the antagonism of insulin and the pituitary to be associated with the posterior lobe. DiBenedetto (1933) found a decreased resistance to insulin in hypophysectomized dogs. Previous injections of anterior lobe extracts increased the resistance to insulin.

The work of Houssay and his followers seems to point to the involvement of the anterior lobe in this carbohydrate relationship whereas the older work of Cushing and his co-workers supports the involvement of the posterior lobe.

Experimental: Insulin tolerance. The dogs were fasted for 18 hours and normal blood sugar taken. They were then given 0.05 cc. of 10U insulin per kilo. body weight. Blood samples were taken every half hour for 8 or 10 hours.

The blood sugar curves of all three dogs reached low points between 2 and 3 hours but returned to normal at different rates. The values for dog I returned to normal only after a little more than 9 hours, and those for dog II after 7 to 8 hours, whereas the values for the control dog returned to normal in 6 hours.

The results demonstrate a definite decreased tolerance to insulin. If the difference in response to insulin of dog I and dog II is at all significant, it is interesting to point out again that dog I had less functioning anterior lobe tissue and it was also more sensitive to insulin. This supports the work of Houssay's group.

Glucose tolerance. The dogs were fasted 18 hours and a normal blood sugar taken. There was no hypoglycemia. One cubic centimeter of 50 per cent sterile glucose per kilo. body weight was injected intravenously at the rate of 8 cc. per minute. Blood samples were taken every 5 minutes

for the first half hour, every 10 minutes for the next half hour and then every 20 minutes for the next hour. Chart 2 shows the results obtained.

The blood sugar values for the control dog were back to normal in 20 to 25 minutes, dog I 40 to 50 minutes, and dog II 70 to 80 minutes, showing a decreased sugar tolerance which is more or less contrary to the findings of others. However, most workers finding an increased tolerance for

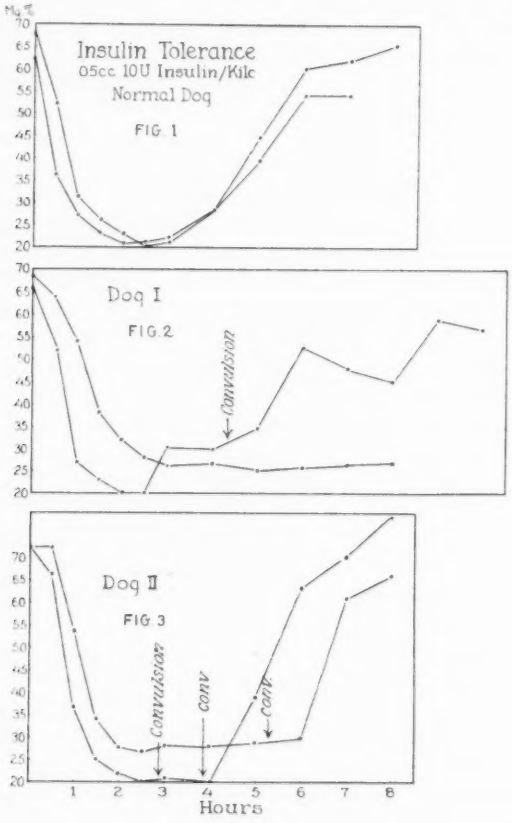

Chart 1

glucose have administered it by mouth. This involves many more factors which, strictly speaking, are not parts of the actual tolerance mechanism. The mouth administration of sugar for glucose tolerance tests has been criticised by many.

Cushing (1912) states that the increased sugar tolerance in long standing cases of gigantism and acromegaly is due to the wearing out of the

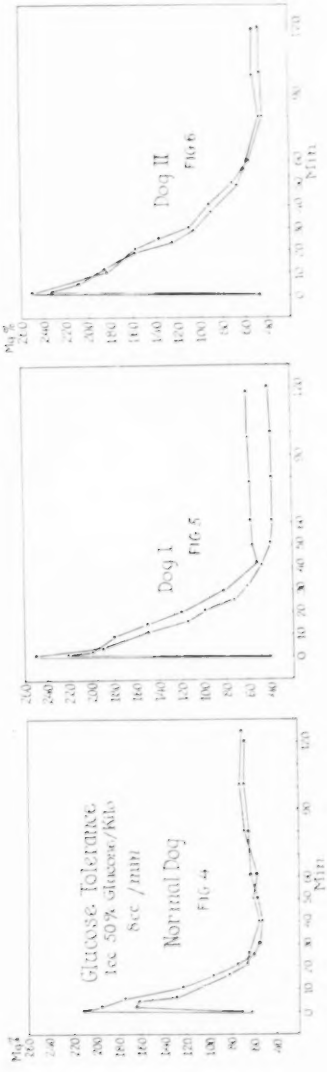
Glucose Tolerance.
1cc 50% Glucose/Kilo
Normal Dog
Fig 4
Dog I
Fig 5
Dog II
Fig 6
Mg%
Min

Chart 2

gland. If it is possible to get a reversion of a hyperactive gland to the hypofunctional state as judged by the response to glucose, does it not seem possible that regenerated anterior lobe tissue might be hyperactive in its antagonism to sugar? If this assumption could be accepted it would

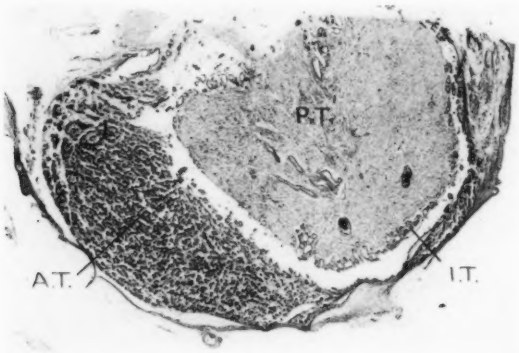

Plate 1. Histological section from dog I. *A.T.*, anterior lobe tissue very loosely arranged suggesting regeneration. *P.T.*, posterior lobe tissue rather normal. *I.T.*, intermediate lobe tissue.

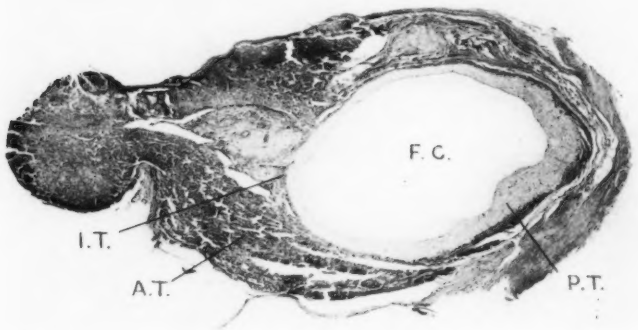

Plate 2. Histological section from dog II. *A.T.*, large amount of anterior lobe tissue with a fairly normal appearance. *P.T.*, posterior lobe tissue. Shows some degeneration at the edge of the fluid cavity. *I.T.*, probably intermediate lobe tissue. *F.C.*, fluid cavity.

explain the decreased sugar tolerances obtained in these dogs where the gland presumably regenerated. The glucose tolerance was less in dog II which had more anterior lobe tissue present.

Autopsy findings. Dog I died of an edema of the lungs shortly after

pancreatectomy, and dog II was killed ten days after pancreatectomy because of its unwillingness to eat and the fact that the wound would not heal. The head of each dog was dissected after injection with formalin. The region about the sella turcica was exposed and gross examination revealed a small cyst-like structure attached to the tuber cinereal region in dog I. In dog II the brain side of the dura revealed what seemed to be a complete hypophysectomy, but imbedded in the bony matrix outside the dura was a small node of what seemed to be glandular tissue. This finding lends more evidence to the regeneration idea since it is quite possible, using the McLean technic, to have a small bit of tissue left in the bony matrix and thus in time see such a growth as was found. Sections were made of the dura and tissue adherent around the sella turcica. Plates 1 and 2 show histological sections from about the middle of the serial sections.[1]

SUMMARY

The extent of hypophysectomy in two dogs is shown by histological sections of the tissue found in and about the sella turcica at autopsy. Functioning parts of all three lobes existed in both dogs. Dog I showed more posterior than anterior lobe tissue remaining while dog II showed more anterior than posterior.

The extent of the lowering of the basal metabolism is probably dependent upon the amount of injury to the tuber cinereal region. It might also be dependent upon the amount of anterior lobe tissue present.

The specific dynamic action of beef heart was not below but rather slightly above normal in these dogs when calculated as calories increase over basal.

Both dogs showed a decreased tolerance to insulin, the degree of decrease being roughly proportional to the amount of anterior lobe tissue present.

The dogs exhibited no hypoglycemia and showed a decreased glucose tolerance.

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[1] We are indebted to Prof. Wilbur K. Smith of the Anatomy Department for the examination of the histological material.

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STUDIES IN THE NORMAL HUMAN WHITE BLOOD CELL PICTURE[1]

II. The Effect of Digestion on the White Blood Cells

JOHN S. LAWRENCE, D. J. STEPHENS and EDGAR JONES

From the Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York

Received for publication July 10, 1933

Since the reports of Nasse (1850) and Moleschott (1854) the prevailing opinion has been that there is an increase in the total number of the circulating white blood cells following the ingestion of food. Certain observers have from time to time questioned such a response on the part of the white blood cells. General reviews of the voluminous literature on this subject have been made by Sabin, Cunningham, Doan and Kindwall (1925) and by Shaw (1927). In recent years there has developed a very strong skeptical attitude toward the occurrence of digestive leucocytosis (Medlar, 1929; Sabin, Cunningham, Doan and Kindwall, 1925; Shaw, 1927; and Smith and McDowell, 1929). Finally Garrey and Butler (1932) have reported observations which have indicated that the low basal leucocyte count of the resting state is unaffected by the intake of large quantities of either protein or carbohydrate.

This communication deals with the effect of the ingestion of a large breakfast on the total number of the white blood cells of normal adults. Our data indicate that the total number of the circulating white blood cells is slightly increased more often than it is unaffected by the ingestion of food.

Methods. Our observations have been made on 15 normal adults. Three women were included in the group. In most cases, the subjects reported at the laboratory at 8:00 a.m. and immediately assumed the recumbent posture. Five of the subjects reported at 7:00 a.m. After the subject had been recumbent for an hour, observations were begun. Total white blood cell counts were then made at 15 minute intervals until 1:00 p.m. The details of the technique were identical with those given in a previous paper (Jones, Stephens, Todd and Lawrence, 1933), two samples of blood being obtained at each interval. Following the obtaining of blood at 9:00 a.m. in the case of the subjects reporting at 7:00 a.m. and at 9:15 a.m. in the case of those individuals reporting at 8:00 a.m., each of the

[1] This investigation was aided in part by a grant from the Josiah Macy Jr. Foundation.

subjects assumed the erect posture, took a few steps to a table, where he sat down and ate leisurely a breakfast consisting of: orange, 100 grams; potato, 150 grams; butter, 20 grams; milk, 20 cc.; tenderloin steak, 240 grams; bread, 50 grams; water (room temperature) as desired up to 500 cc. As soon as breakfast was ingested (15 to 30 min.) the subject lay down again and remained recumbent until the end of the period of observation. The only exceptions to this were that the subjects arose to urinate when this was desired. One individual defecated during this period.

Seven of the subjects had similar observations made at another time when they were given no breakfast and kept under basal conditions. Four of these six subjects had exactly similar observations made on their blood during normal activity in the laboratory both with and without breakfast. Fixed smears were made at 15 minute intervals on four of the subjects. These smears were stained with Wright's stain, after which Schilling differential counts of 200 cells were made.

Results. There was a distinct but not marked increase in the total number of white blood cells in the peripheral blood of three individuals after the ingestion of food as compared with values obtained in these same subjects when under basal conditions, no food being allowed. There was no demonstrable increase following the ingestion of food in four additional individuals who had had other sets of observations made under basal conditions.

Six of the eight individuals who had only one series of observations, namely, after the ingestion of food, showed an increased number of white blood cells as compared with the figures which we have previously shown (Jones, Stephens, Todd and Lawrence, 1933) would be expected under similar conditions without food. The remaining two individuals in the group showed no change in the number of leucocytes that could be ascribed to the ingestion of food. In summary, it is seen that there were nine instances in which an increase in the total number of the white blood cells occurred after the ingestion of food and six when there was no change in the total number of leucocytes that could be attributed to food. The intervals after the beginning of breakfast before the increase was noticeable were: in one subject, $1\frac{1}{4}$ hours; in four subjects, $1\frac{1}{2}$ hours; in three subjects, 2 hours, and in the remaining subject, $2\frac{3}{4}$ hours. In all of these subjects this increase persisted to the end of the period of observation but in one of them there was a temporary drop in the number of the cells 2 hours after breakfast. There was a general tendency for the curve representing the total number of the cells to rise gradually from the time of the first increase in all but one of these six subjects (C. W.). In this latter subject the curve remained more or less constant after the initial increase. The total increase in the number of the cells was sufficiently great in only one instance, (D. S.) to produce a value as high as 10,000 per cu.mm., the highest figure

for the maximum values in this group with this one exception being 9575 per cu.mm. and the lowest figures for the maxmium values being 6875 per cu.mm. The differences between the highest and lowest figures in each of the 15 individuals for the entire period ranged from 1600 per cu.mm. to 5750 per cu.mm., with an average of 3249 per cu.mm. Corresponding figures for 9 basal recumbent subjects were 1249 to 3300 per cu.mm., with an average of 2137 per cu.mm. (Jones, Stephens, Todd and Lawrence, 1933).

Analysis of the results obtained when four of the subjects were allowed normal activity shows that there are so many fluctuations which *may* occur as a result of activity that the slight increase from the ingestion of food may not be observed. In no single instance could a definite increase in the number of leucocytes following the ingestion of food be demonstrated in the four subjects allowed normal activity. Two of these subjects showed very marked fluctuations when counts were made on them while carrying on their ordinary duties in the laboratory. Thus, the totals in one subject fluctuated between 13,825 and 8750 per cu.mm. whereas similar figures in the other subject were 10,725 and 5600 per cu.mm. The other two subjects, who had counts made during activity, showed curves with normal activity without breakfast, that were entirely comparable with those obtained under basal conditions.

Chart 1 represents the curves for the total number of white blood cells in one subject who had observations made on four occasions. The first curve represents the findings under basal conditions; the second gives the values obtained when recumbent with the addition of food; and the third and fourth curves give the findings when active without food and when active with food respectively.

The Schilling differential counts, after the ingestion of food, revealed the same response on the part of the individual white blood cells as was found in the basal subjects (Jones, Stephens, Todd and Lawrence, 1933). In other words, the neutrophils accounted in a large measure for the increase in the total number of the cells.

Discussion. Our data indicate that a definite but not marked increase in the total number of the circulating white blood cells often follows the ingestion of food. This is not a constant reaction but, in our series, has occurred in nine out of fifteen subjects. It is of interest that $1\frac{1}{4}$ to $2\frac{3}{4}$ hours elapse between the ingestion of food and the initial increase in the total number of leucocytes. This increase is not transient but may be detected up to 4 hours after the intake of food. We have not extended our observations further and, hence, do not know how long this reaction lasts. Five hours of continuous observation have seemed to us long enough for any one subject as the procedures may become distinctly tiring after this interval.

No serious attempt has been made to determine what type of food is responsible for the changes in the number of the white blood cells. Whether

this response is non-specific cannot be said. A priori, one would consider that the response was not due to carbohydrates since it occurs after the time of maximum carbohydrate absorption. In view of the negative results of Garrey and Butler (1932) with high protein and high carbohy-

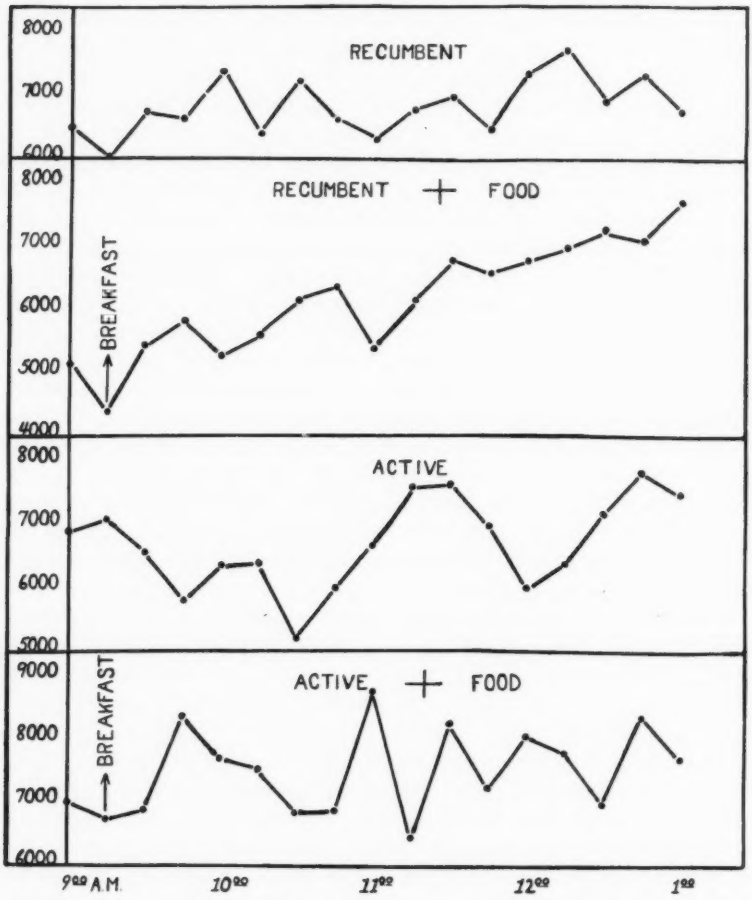

Chart 1. Curves representing the total number of white blood cells in the peripheral blood of one subject under varying conditions at different times.

drate meals, the fat content of our meals may have been the responsible factor. However, this was not high as only 20 grams of butter were given. This is the only possible explanation which we have been able to evolve for the differences between our results and those of these authors.

It will be noticed that we allowed each of our subjects to assume the sit-

ting posture while eating. Further, each of these individuals was allowed to walk from the bed to the table upon which his food was placed. We have been unable to detect any effect of such procedures on the shape of the curve representing the total number of the white blood cells. This, of course, is in conformity with the observations which we have reported previously on the effect of short periods of mild exercise (Jones, Stephens, Todd and Lawrence, 1933).

We have no satisfactory explanation for the failure of response in six of the subjects in this series. It may be that there is an individual difference in the type of response which is obtained.

It is of interest that the differential counts on four of the subjects showed the same response on the part of the different type of cells as was found in normal basal subjects (Jones, Stephens, Todd and Lawrence, 1933). Thus, the increased number of all the white blood cells was due to an increase mainly in the neutrophils.

The failure to demonstrate changes in the number of the white blood cells following the ingestion of food by individuals allowed normal activity is of interest. This is in conformity with the reports of Shaw (1927) and of Sabin, Cunningham, Doan and Kindwall (1925).

From a clinical point of view, the effect of food on the white blood cell count is not of a great deal of importance since the increase was not great and, in every instance except one, the total number of the cells was less than the ordinarily accepted upper limit of 10,000 per cu.mm. In round figures, food would never be expected to increase the total count above 2000 to 3000 per cu.mm. From a purely theoretical point of view, since basal white blood cell counts will show less variation than others, it would seem that these would be most reliable. However, it seems to us that the inconveniences necessitated by the introduction of such procedures in a clinic far outweigh the advantages to be gained.

CONCLUSIONS

An increase in the total number of white blood cells in the peripheral blood of normal adult subjects has been shown to occur in the majority of instances following the ingestion of food. This increase is not of sufficient magnitude to be of clinical importance.

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THE EXCRETION OF CAESIUM BY THE ALBINO MOUSE

CLARENCE F. GRAHAM AND ARTHUR W. WRIGHT

From the Bender Hygienic Laboratory, Albany, N. Y.

Received for publication July 8, 1933

In an investigation of the effect of caesium chloride upon tumor growth in mice, the writers recently had occasion to ascertain the rate at which caesium is excreted by these animals, but a search of the literature disclosed no quantitative determinations which would give the desired information. Hanford (1) some years ago found that caesium is retained in the tissues for at least 10 days after its administration and that it is excreted in the urine and feces by cats, dogs and rabbits, but he did not determine the relative amounts excreted through the kidney and the intestine, and the estimations were not quantitative. The work here reported was undertaken to determine the rate at which injected caesium is excreted by the albino mouse, and the relative amounts eliminated in the urine and the feces.

At first an attempt was made to determine the caesium in the excreta by direct chemical methods, but the effort was unsuccessful because the difficult fractional precipitation and crystallization of the caesium salt could not be carried out with the small amount of material available. A spectroscopic method was therefore adopted based on the technique of Gooch and Phinney (2), using the combined caesium lines at 4555.3 and 4593.2 A.U., which appear as a single line in a spectroscope of moderate dispersion. The original method calls for a comparison of the brightness of two spectra produced in quick succession by the introduction of the unknown solution and a solution of known strength into the flame on a coil of platinum wire. It was found that the brightness of caesium spectra could not be compared with any approach to accuracy, but that the dilution at which the combined caesium lines could last be seen repeated itself with fair accuracy when the same amount of caesium was present in solutions of the same character. Since digestion extracts from urine and feces contain inorganic salts in considerable amounts, the caesium-containing extract was compared with a blank extract from excreta collected for the same period and prepared in the same way. The extract containing caesium was diluted with water until the caesium line disappeared, the blank extract was then diluted to the same amount and caesium added to it until the line could first be obtained. The amount of caesium added to the blank extract compared to the amount injected into the mice gave the excretion ratio for the collection period.

EXPERIMENTAL METHOD. The mice were confined in a glass jar on bread and water for a 24-hour collection period immediately after the subcutaneous injection of 1.0 cc. of an aqueous solution containing 0.026 gram of caesium chloride, which is isosmotic with 0.9 per cent NaCl solution. The extract for spectroscopic examination was prepared in either of two ways which gave practically the same figures for the excretion rate. The excreta, washings of the jar, and any food in the food cup were digested with either nitric or sulphuric acid on a sand bath at low heat, thus converting the excreted caesium salts into the nitrate or the sulphate. If nitric acid is used, the material must not be allowed to become dry and overheated or the volatile caesium nitrate will be lost, but the sulphuric acid digestion can be carried to dryness and charring, since the sulphate does not volatilize even at low red heat. After digestion, the concentrated liquid or carbonized mass was mixed with water and washed through a filter paper, the filtrate being used for spectroscopic examination. To eliminate errors in the measurement of the small quantities involved, the addition of caesium to the blank extract was always made with the same syringe used for the injection, calibrated to 0.05 cc., and the same caesium chloride solution injected was also added to the extract.

Some difficulty was experienced at first in judging the disappearance of the caesium line because of the strong flare set up in the spectroscope field by the sodium salts in the solution. The insertion between flame and slit of a Wrattan H45 filter, passing the band 4200 to 5400 A.U., eliminated this disturbance and gave a dark blue field on which the caesium line showed up clearly. Another uncertainty arose from the varying sensitivity of the observer's retina during the period of observation while the solution was being diluted. In order to prevent progressive dark adaptation and change in retinal acuity, a three candlepower electric lamp was arranged to throw a diffuse light onto the prism so that it would be reflected into the telescope. This light was colored orange yellow by the insertion of Wrattan filters E22 and G15 in the path of the beam, giving a color roughly complementary to that of the caesium line. A depression switch on the vertical support of the spectroscope allowed the light to be turned off just as an observation was to be made, and a rheostat controlled the intensity of the light, which was kept constant during any period of observation. The same loop of platinum or Nichrome wire was used in each series of observations, the slit was fixed at 0.1 mm. throughout the experiments, and a Bunsen burner was rigidly attached to the spectroscope with its 3.0 cm. flame 2.5 cm. from the slit.

QUANTITATIVE RESULTS. With these refinements the percentage excretion of caesium was determined on a considerable number of extracts of excreta. The excretion of caesium in the 24-hour period following the injection of 0.026 gram of caesium chloride was found to vary between 17.5

per cent and 20 per cent of the amount injected. The setting of the spectroscope prism, the brightness of the dark adaptation light, the manner of preparing the extract, and the method of dilution were altered in different ways with an extreme variation of 2.5 per cent between the highest and the lowest figures.

Instead of the blank extract of excreta, other solutions were also used for comparison with the caesium-containing extract of excreta from injected mice. Water, 0.015 per cent NaCl solution, 0.03 per cent NaCl solution, and water acidulated with sulphuric acid gave caesium excretion percentages of 17.5 per cent to 20 per cent when tested against the extracts of excreta from animals which had been given injections of caesium chloride.

The excretion of caesium in the second 24 hour period following the injection was determined in the same way for a single mouse and for four mice in one jar, and was found to be 16 per cent and 15 per cent of the first 24 hour amount in the two cases, or 3 per cent of the caesium originally injected.

The excretion could not be determined beyond the second 24-hour period on account of the small quantities of caesium involved, but traces of caesium were found in the bodies of the mice as late as the tenth day after injection, but not later.

Relative amounts of caesium excreted in the urine and the feces. For a determination of the relative amounts of caesium excreted in the urine and the feces, a special cage was devised which made the separation of the excreta comparatively easy. A metal cylinder 10 cm. in diameter was provided with a wire bottom of 9 mm. mesh, and a piece of soft filter paper was supported below the wire bottom at a distance of about 7 mm. so that the mice could not reach the paper and tear it to pieces. A 12 candlepower carbon filament lamp was placed about 10 cm. below the filter paper so that its heat would dry out the excreta promptly, and after the mice had spent 24 hours in the cylinder the feces were picked off the paper with forceps for digestion, while the paper and cage washings were digested to give the extract of urine.

On a bread and water diet, the mice excreted 15 per cent to 16 per cent of the injected caesium in the feces, and 84 per cent to 85 per cent in the urine.

These figures are an interesting comparison to those obtained for the excretion of rubidium by Mendel and Closson (3) who found that the first day excretion of rubidium by the rabbit, following the injection of 285 mgm. RbCl per kilo, was divided between feces and urine in the proportion of 5 per cent to 95 per cent. In a 19 day period the feces contained only about 2 per cent of the excreted rubidium. Comparable results for the dog and cat indicated that only a small percentage of rubidium is excreted by the intestine. Lithium also, according to Good (4), is excreted pre-

dominantly by the kidney, and only to a slight extent by the intestine. The alkali earths strontium and barium, however, are excreted almost exclusively by the intestine, as shown by Mendel and Thacher (5), and by Mendel and Sicher (6).

Caesium, therefore, with a kidney to intestine excretion ratio of 5.6 to 1, is intermediate between the other alkali elements and the alkali earths in its mode of excretion.

SUMMARY

1. A spectroscopic method is described for the determination of small amounts of caesium in excreta.

2. In the 24 hour period following the subcutaneous injection of 0.026 mgm. CsCl in a solution isosmotic with 0.9 per cent NaCl solution, albino mice excrete from 17.5 per cent to 20 per cent of the injected caesium.

In the second 24 hour period, the excretion is 15 per cent to 16 per cent of the first 24 hour amount, or 3 per cent of the caesium originally injected.

3. Albino mice excrete 84 per cent to 85 per cent of the injected caesium in the urine and 15 per cent to 16 per cent in the feces.

4. In its mode of excretion caesium is intermediate between the other alkali elements and the alkali earths.

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THE K-CA ANTAGONISM IN REGARD TO ABSORPTION FROM THE INTESTINE

ERNST GELLHORN AND ARTHUR SKUPA

From the Department of Physiology, College of Medicine, University of Illinois, Chicago, Illinois

Received for publication July 3, 1933

In several papers of this series (Gellhorn and Northup, 1933a, b; Gellhorn, 1933c) it has been shown that hormones like adrenalin, insulin, thyroxin and others influence the absorption of glucose from the gut under conditions in which the perfusion rate of Ringer's in the blood vessels is kept constant. These results were interpreted as due to changes in the permeability of the gut membrane. If this interpretation is correct, it is probable that the permeability of the gut membrane could be greatly altered by changing the ionic composition of the perfusion fluid in the blood vessels, since ions are perhaps the most efficient reagents which alter cellular permeability. Moreover, the two problems as to the effect of hormones and of ions on permeability and absorption may be interrelated since not infrequently it has been noted that injections of various hormones alter the ionic content of the tissues (compare, e.g., Dresel and Wollheim, 1924, and Sugimoto, 1932). For these reasons a study of the effects of K and Ca on absorption was undertaken. In the present paper this study is restricted to the influence of these ions on absorption when their concentration in the vascular perfusion fluid is altered. A few qualitative observations on the effect of K and Ca on the excretion of the dyestuff cyanol through the gut wall were reported by Mond (1924).

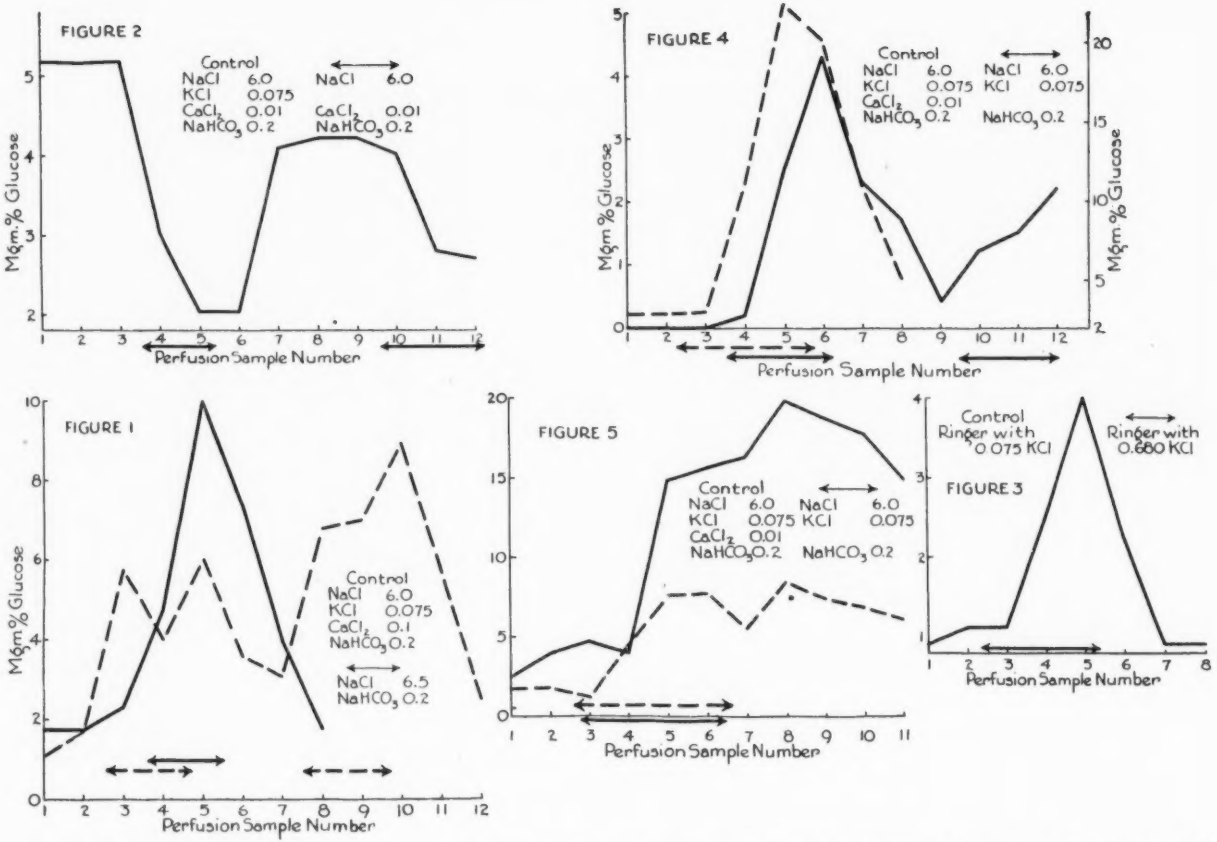
The method consisted in the separate perfusion of the blood vessels supplying the gut of a frog (Rana esculenta) and of the gut itself. The latter was perfused with 3.15 per cent glucose solution, the former with Ringer's solution of various compositions as detailed in the results. The glucose which was absorbed into the Ringer's solution was determined by the Folin-Wu method, modified to suit the conceatrations found, a photoelectric cell being used as a colorimeter. The various solutions with which the blood vessels were perfused were without influence on the diameter of the blood vessels. Therefore the perfusion rate remained constant throughout the entire experiment, thus making pressure adjustments unnecessary. Since the perfusion pressure remained practically unchanged, its reproduction in the figures is omitted. As to details in

this method, originally devised by Mond, compare the previous papers of this series. Sixty-four experiments were performed.

RESULTS. Before entering into a discussion of the results obtained with solutions in which the K or the Ca content of the Ringer's solution was altered we wish to mention one series of experiments in which the absence of both K and Ca on the absorption was studied. A typical example is reproduced in figure 1. It shows that if we exchange Ringer's solution for one without KCl and $CaCl_2$ the permeability to glucose invariably increases. The effects are reversible and can be repeated in the same preparation as the interrupted line in figure 1 indicates. This experiment is in good agreement with experiments done with various plant and animal cells in which it was shown that equilibrated salt solutions produce a state of lower permeability than is observed if by leaving out one or more constituents of a balanced solution this balance is altered (compare Gellhorn, 1929). It may be mentioned that the pH and the osmotic pressure are kept constant.

The next task was to analyze the importance of K and Ca separately. Particular attention was paid to the question whether or not the K and Ca concentrations ordinarily present in Ringer's solution are of physiological significance in regard to absorption. Twenty-six experiments were performed in which the effect of various KCl concentrations was investigated. It was found that under optimal conditions a KCl concentration of 0.0075 per cent displays a powerful effect on the permeability of the gut. As shown in figure 2, the absence of this small amount of KCl considerably reduces the adsorption rate of glucose. The effect is reversible and can be studied several times in the same preparation.

Experiments were carried out on the effect of low concentrations of KCl in the presence of various $CaCl_2$ concentrations. It was generally found that the effect of small KCl concentrations is more distinct at low $CaCl_2$ concentrations than at high ones. As the experiments to be described presently will show, $CaCl_2$ decreases the permeability of the gut to glucose. In several hundred experiments in which the frog gut preparation has been perfused in our laboratory during the past two years we frequently observed that the amount of sugar which permeated through the gut gradually declined. In some cases we have had to discard preparations because the amount of glucose in the capillary perfusion fluid was zero. It was now found that preparations with a very low sugar concentration in the capillaries, i.e., with a very low permeability of the gut to glucose, were often rather refractory to ion effects which ordinarily bring about an increase in permeability. The reason seems to be a cumulative effect of $CaCl_2$ sufficient to suppress the effect of an increased KCl concentration. Even under these conditions however the KCl effect brings about a reversible increase in permeability of the gut to KCl provided that the KCl concentration is high enough (fig. 3).



Figs. 1–5. The curves represent the glucose concentration in the blood vessels under the influence of various salt solutions with which the blood vessels were perfused. The composition of these salt solutions (for 1000 cc.) is indicated on each graph. The gut was regularly perfused with 3.15 per cent glucose. In figure 3 the $CaCl_2$ content is 0.02 per cent. Dash line indicates a duplicate experiment.

It has just been stated that $CaCl_2$, in concentrations ordinarily used in Ringer's solution, seems to exert a cumulative effect on the gut permeability, resulting in a decreased permeability to glucose which does not wear off immediately after the Ringer's solution has been exchanged for a $CaCl_2$ free solution. Therefore no change in glucose absorption may occur under these circumstances. If, however, a Ringer's solution with the same $CaCl_2$ and a higher KCl concentration is employed (e.g., 0.020 per cent $CaCl_2$ and 0.021 per cent KCl) the exchange of the $CaCl_2$ containing solution for $CaCl_2$-free Ringer's leads to a considerable and reversible increase in the gut permeability to glucose, as table 1 indicates.

It is interesting and is in agreement with our interpretation of the cumulative effect of 0.02 per cent $CaCl_2$ in Ringer's solution, that by using this

TABLE 1

PERFUSION SAMPLE NUMBER	PERFUSION FLUID	AMOUNT OF PERFUSION FLUID	GLUCOSE CONCENTRATION
		cc.	*mgm. %*
1	I*	3.4	0.0
2	I	3.5	0.4
3	II	3.6	1.7
4	II	3.5	0.9
5	I	3.7	0.2
6	I	3.7	0.0
7	II	3.9	0.6
8	II	3.8	0.9
9	II	3.6	1.7
10	I	3.8	0.9
11	I	3.8	0.0

*I =		II =	
NaCl	6.00	NaCl	6.00
KCl	0.21	KCl	0.21
$CaCl_2$	0.20	$NaHCO_3$	0.20
$NaHCO_3$	0.20	Dist. wat. ad	1000.0
Dist. wat. ad	1000.0		

salt in a much smaller concentration very considerable effects on glucose permeability are noted. As figure 4 shows, even the change from 0.001 per cent $CaCl_2$, which is about $\frac{1}{20}$ of the $CaCl_2$ concentration ordinarily used in Ringer's solution, to Ca-free Ringer's brings about a very strong increase in glucose absorption. Figure 5 shows that if the $CaCl_2$-free solution is employed for a little longer (during four periods) the increase in permeability is much prolonged, so that even after four periods in $CaCl_2$-containing Ringer's solution the gut permeability has not returned to its earlier level.

These experiments seem to show that K and Ca influence the permeability of the gut wall to sugar in a fashion similar to their effects on the

permeability of other cells. K increases and Ca decreases permeability even in concentrations which are smaller than those ordinarily employed in Ringer's solution. From the fact that in the presence of 0.01 and 0.02 per cent $CaCl_2$ a decline in the glucose concentration not infrequently occurs, which is absent in experiments with smaller $CaCl_2$ concentrations (0.001 and 0.005 per cent), it is concluded that the latter solutions are preferable for a study of ions and other reagents on the permeability of the gut wall. It is interesting to note that no edema of the gut wall occurred even in the perfusion experiments with very low $CaCl_2$ concentrations since the perfusion pressure remained low and practically unaltered for two to three hours.

SUMMARY

1. In the absence of both K and Ca as present in Ringer's solution the permeability of the gut to glucose increases reversibly.

2. Slight changes in the concentration of KCl and $CaCl_2$ in Ringer's solution with which the blood vessels supplying the gut are perfused lead to changes in the permeability of the gut wall to glucose. The absorption of glucose is increased by potassium and decreased by calcium. The effects were observed even with solutions containing only 0.0075 per cent KCl and 0.001 per cent $CaCl_2$. Since $CaCl_2$ in concentrations of 0.01 to 0.02 per cent seems to have a cumulative irreversible effect on the gut wall frequently leading to a gradual decrease in glucose absorption, the perfusion with a Ringer of lower $CaCl_2$ concentration (0.001–0.005 per cent) is preferable. The perfusion pressure necessary to maintain a constant perfusion rate remains practically unchanged throughout the entire experiment.

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NOTE ON THE FAILURE OF ANTERIOR LOBE EXTRACT TO PASS FROM MOTHER TO FETUS IN RABBITS AND CATS

LEROY GOODMAN AND GEORGE B. WISLOCKI

From the Departments of Anatomy and Obstetrics, Harvard Medical School, Boston

Received for publication July 13, 1933

It is now a well-established observation that a mature female rabbit can be made to ovulate by the administration of extracts of the anterior hypophysis or of the hypophyseal-like substance contained in human urine of pregnancy. We have made this observation the basis of investigating whether or not these hormonal substances are transmitted through the placenta from mother to fetus in rabbits and cats.

In the experiments six pregnant rabbits and seven pregnant cats were used. To these animals hypophyseal hormone (Antuitrin G) or the active substance of pregnancy urine (Antuitrin S) was administered.[1] At varying periods subsequent to the injections the animals were killed, the uterus opened and the fetal fluids collected. The fluids so obtained were then injected into mature, female rabbits. The ovaries of these test animals were examined two days later to see whether or not ovulation had occurred. The results of the two series of experiments are set forth in tables 1 and 2.

It will be seen from table 1 that the injections into the pregnant rabbits were all carried out between the twenty-first and twenty-fifth day of gestation. This is the period at which the amount of amniotic fluid is at its maximum in the rabbit (Lell, 2). The hypophyseal hormone was administered in one large dose with one exception in which the dose was divided and given over a period of twelve hours. The pregnant rabbits were opened twenty-four hours later, excepting two which were killed at nine and twelve hours respectively. The ovaries of the mother in each instance showed recent ovulation points. The fetuses were examined in every instance to see that they were living and that there was no separation of the placenta. The amniotic fluid was collected and pooled from the several fetuses. Varying amounts of the amniotic fluid were then injected into mature, isolated, test rabbits. Forty-eight hours later these were opened to see whether or not the amniotic fluid had caused ovulation. In no instance was ovulation produced. Hence, we may conclude that under the

[1] We are indebted to Dr. E. A. Sharp of Parke, Davis & Co. for the necessary supply of Antuitrin G (anterior hypophyseal extract of beef) and Antuitrin S (concentrated human urine of pregnancy).

TABLE 1

Effects on ovaries of rabbits of injecting amniotic fluid obtained from pregnant rabbits which had received injections of Antuitrin G or S

PREGNANT RABBITS	DOSE OF HORMONE. MODE OF ADMINISTRATION	OVARIES OF PREGNANT RABBIT	TIME ELAPSED BEFORE REMOVAL OF AMNIOTIC FLUID	QUANTITY OF AMNIOTIC FLUID INJECTED INTO TEST RABBIT	RESULTS IN TEST RABBITS
			hours	*cc.*	
1. 21 days	10 cc. Antuitrin G, intravenously in 1 dose	Ovulation	12	12	No ovulation
2. 21 days	10 cc. Antuitrin G, intravenously in 1 dose	Ovulation	24	3	No ovulation
3. 21 days	10 cc. Antuitrin G, intravenously in 1 dose	Ovulation	24	10	No ovulation
4. 21 days	10 cc. Antuitrin G, intravenously in 1 dose	Ovulation	24	10	No ovulation
5. 25 days	10 cc. Antuitrin S, intravenously in 3 doses	Ovulation	9	27	No ovulation
6. 25 days	10 cc. Antuitrin G, intravenously in 1 dose	Ovulation	24	10	No ovulation

TABLE 2

Effects on ovaries of rabbits of injecting allantoic and amniotic fluid obtained from pregnant cats which had received injections of Antuitrin G or S

PREGNANT CATS. C. R. LENGTH OF FETUSES	DOSE OF HORMONE. MODE OF ADMINISTRATION	OVARIES OF PREGNANT CAT	TIME ELAPSED BEFORE REMOVAL OF FETAL FLUIDS	QUANTITY OF FETAL FLUIDS INJECTED INTO TEST RABBITS (A) AMNIOTIC FLUID (B) ALLANTOIC FLUID	RESULTS IN TEST RABBITS
			hours		
1. 43 mm	4 cc. Antuitrin G, intravenously in 1 dose	No ovulation	48	10 cc. of mixture	Ovulation
2. 50 mm	1 cc. Antuitrin S, daily for 12 days subcutaneously	Ovulation	24	(a) 4 cc. (b) 10 cc.	No ovulation
3. 50 mm	10 cc. Antuitrin G, intravenously in 1 dose	No ovulation	12	(a) 10 cc. (b) 10 cc	No ovulation
4. 62 mm	11 cc. Antuitrin G, intravenously in 2 doses 24 hrs. apart	No ovulation	4	(a) 10 cc. (b) 10 cc	No ovulation
5. 80 mm	10 cc. Antuitrin G, intravenously in 1 dose	No ovulation	8	(a) 4 cc. (b) 10 cc.	No ovulation
6. 100 mm	2 cc. Antuitrin G, daily for 6 days subcutaneously	No ovulation	24	(a) 6 cc. (b) 10 cc.	No ovulation
7. 130 mm	10 cc. Antuitrin G, intravenously in 1 dose	No ovulation	48	(a) 5 cc. (b) 10 cc.	No ovulation

conditions of our experiments the hormonal substance had not been transmitted from the mother to the amniotic fluid of the fetuses. It might be added that the Antuitrin G and S preparations which we were using were of adequate potency, for assayed upon non-pregnant rabbits we produced ovulation invariably with 0.1 cc. Thus the dose which we were giving to our experimental animals was, as a rule, at least one hundred times the minimal ovulating dose.

In table 2 are set forth the results of similar experiments in pregnant cats upon the transmission of Antuitrin G and S from mother to fetus.

The procedure on the pregnant cats was essentially the same as in the preceding series of pregnant rabbits. However, in the cats there were two fetal fluids—the allantoic as well as the amniotic fluid—to be tested. The pregnant cats represent a series from about the middle of pregnancy on. The fetal fluids are maximal in quantity, excepting that from the 60 to 80 mm. stage on the amniotic fluid begins slowly to decline. Exact data, however, on the amounts of these fluids at different stages in the cat are lacking, and our observations on this point may be considered merely as approximate. With one exception our results in the cat were negative for both allantoic and amniotic fluids. We have no explanation to offer for the one positive result. Whether it is a result of faulty technique or due to unknown factors which our experiments do not elucidate, we are unprepared to say.

Attention should be called to the fact that the pregnant rabbits which served as donors all ovulated. In contrast to the rabbits the pregnant cats of our present series (with one exception) did not ovulate with either repeated large doses or a single large dose of the antuitrin preparations. This is in keeping with previous experiences (Snyder and Wislocki, 5) upon the failure of ovaries of cats to respond by ovulation, except in rare instances, to injections of anterior lobe hormone or urine extract. Of a total of eleven pregnant cats to which we have at various times given large, single or repeated doses of Antuitrin G or S, only two have ovulated. We should add to this account the observation that large doses of Antuitrin S have caused several of our pregnant rabbits and cats to abort. These animals were discarded for our present purposes. In two cats near term abortion occurred on the 4th and 7th days after daily injections of 2 and 1 cc. of Antuitrin respectively.

Discussion. It appears, therefore, that under the conditions of our experiments the hormonal extracts which we were using did not reach the fetal fluids of either the rabbit or the cat. The placenta and the fetal membranes appear to constitute for these substances an impermeable barrier between the maternal circulation and the amniotic and allantoic fluids. The rôle played by the respective parts of this barrier in failing to allow the hormonal substances to pass is a complicated question to

answer. There have been two main views concerning the formation and sources of the amniotic fluid—one, that it is a maternal transudate; the other, that it is fetal in origin arising either as a transudate or a secretion. The origin of the allantoic fluid is also subject to uncertainty, the most widespread opinion holding that it is largely or entirely fetal and derived to a considerable extent from the activity of the fetal kidneys. Many of the discordant views concerning these matters may be traced, in our estimation, to the fact that practically no attention has been paid in the past to several important factors, namely, the changes in quantity and character of the fluids in a given species of animal during the different stages of gestation, as well as the wide morphological variability of the membranes to the uterus in various classes of mammals. These factors undoubtedly influence the mode of formation of the fetal fluids within different classes of animals, as well as at different stages of the gestation period in the same species.

In the rabbit and cat in the second half of gestation, the period with which we are dealing, it can be postulated with a certain degree of likelihood that the fetal fluids of these animals are exclusively fetal in origin. In favor of this interpretation two anatomical considerations lend weight. The first is that the chorion of the cat and the vitelline membrane of the rabbit which are respectively the membranes which enclose the fetus and its attendant fluid-containing membranes, are amply vascularized by fetal vascular beds which are interposed between the fetal fluids and the uterus. The second consideration is that in the cat and rabbit the epithelial limiting surface of the endometrium is rather completely restored, excepting at the placental site, during the second half of gestation. These two considerations rather preclude for the rabbit and cat the direct entrance of maternal transudate into the allantoic and amniotic fluids. If these arguments have validity, then the fetal fluids in these two animals are presumably of fetal origin and the only means of ingress to them of substances present in the mother would be by way of the placenta and the fetal circulation. By what actual areas of the fetal vascular beds the fluids themselves are elaborated remains open to conjecture. In spite of these uncertainties our observations indicate that gonado-kinetic hormonal substances introduced into the maternal circulation of the cat and rabbit do not reach the fetal fluids, and this is due presumably to a failure of the hormonal substances to enter the fetal circulation through the placenta.

Recent work of a similar nature upon placental permeability towards hormones appears to corroborate our results. Wislocki and Snyder (6) published not long ago a note upon the failure of anterior lobe extract to pass in the rabbit in the opposite direction, namely, from fetus to mother. Furthermore, in recent papers Snyder and Hoskins (4) and Hoskins and Snyder (1) have reported the failure of transmission from

fetus to mother through the placenta of the rabbit of other active principles (adrenalin, insulin, pituitrin, parathyrin).

Concerning the relative permeability of placentae of various mammals, it may be stated in brief, from the morphological work of Grosser upon the number of layers (constituting the placental barrier) intervening between the maternal and fetal circulations, as well as from numerous studies upon relative placental permeability, that the endotheliochorial placenta of the cat is considered to be less permeable than the hemochorial placenta of the rabbit or man. In reference to the two latter, their permeability is on functional grounds considered to be about the same, although recent morphological work by Mossman (3) indicates that in the rabbit the barrier between the maternal and fetal circulations may be more tenuous and consist of one less layer than in man. Be that as it may, the degree of placental permeability in man and the rabbit is from all indications of about the same order, so that experimental results obtained in one might be regarded as in some measure applicable to the other.

It is interesting to note that our negative experiments, besides testing the passage of anterior lobe substance, also demonstrate that in the amniotic fluid of the rabbit and cat, as well as in the allantoic fluid of the cat, there is normally no substance present capable of producing ovulation in the rabbit. This adds substantiating evidence that gonado-kinetic substances are not demonstrable in the fetal fluids outside of man and possibly the great apes.

The reason, however, for the presence normally of anterior lobe-like substance in the amniotic fluid and fetal urine of man is obscure. One must assume either that it is elaborated in the placenta or that it is formed in the mother and transmitted through the placenta to the fetus. Its presence in the amniotic fluid might conceivably be accounted for by the fact that the chorion laeve of man is avascular and is, moreover, rather intimately fused with a modified and necrotic endometrium, the decidua vera. These circumstances might render the chorion and the adjacent amnion directly permeable to some extent to the substances present in the mother without the necessity of their traversing first the placenta and entering the fetus to reach the amniotic fluid. However, the normal presence of the gonado-kinetic hormone in fetal human urine cannot be readily accounted for without assuming some activity on the part of the placenta. The fetus itself can be ruled out as a possible locus of origin of the hormone because of the rapid disappearance of the substance from the fetal urine after parturition. Thus we are faced with accepting the placenta as involved in its formation or transmission in man. The present experiments on rabbits and cats suggest that it is not readily transmitted by the placenta and offer the solution that in man the hormonal substance may be actually elaborated in the placenta thus accounting for its access

to the fetus. In spite of this reasoning, factors of dosage and the duration of time in our experiments may be so different from conditions prevailing in human pregnancy that one would not be justified in drawing any final conclusion from them in regard to the human.

SUMMARY

1. Amniotic fluid of the rabbit and amniotic and allantoic fluids of the cat injected into mature rabbits do not cause ovulation.

2. Antuitrin G and S (extracts respectively of the anterior lobe of the hypophysis of cattle and of human urine of pregnancy) are not transmitted from the maternal blood stream to either the amniotic fluid of the pregnant rabbit or the amniotic or allantoic fluids of the pregnant cat.

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AN ANALYSIS OF THE FACTORS CONCERNED IN THE AUTOMATIC ADJUSTMENT OF THE VENTRICLE TO INCREASED LOAD

I. A Study of the Importance of Contraction Rate and Filling Pressure in Producing Alterations in Diastolic Volume by Variations in Arterial Pressure*

LEON F. MOLDAVSKY and MAURICE B. VISSCHER

From the Department of Physiology, College of Medicine, University of Illinois, Chicago, Illinois

Received for publication July 13, 1933

It has long been known that the ventricle under ordinary conditions responds to an increase in work imposed upon it by an increase in the diastolic volume. Starling and Visscher (1927) showed the physiological significance of this mechanism in connection with the energy liberation of the heart in contraction. They established the fact that the magnitude of energy liberation in contraction is dependent upon the length of the fibers of the ventricle at the end of diastole. It therefore became apparent that the automatic mechanism of the heart in responding to an increased load serves to bring about a spontaneous increase in energy liberation with which to carry out the augmented work thrown upon it. It is the object of this investigation to show upon what factors the automatic adjustability of the ventricle to increased pressure load depends.

The change in diastolic volume of the ventricle produced by an alteration in arterial pressure against which the ventricle must pump has therefore been investigated in relation to the heart rate, the filling pressure and the temperature. It has been observed in these experiments that below a certain critical heart rate and above a critical filling pressure, the arterial pressure has no influence upon the diastolic volume of the ventricle. This obviously indicates a set of limiting conditions for the application of the "law of the heart" indicated by Starling (1915). The observations reported here, as will be seen, demarcate the limits within which this mechanism of adaptation is operative, and demonstrate that this so-called law is a description of the behavior of the ventricle under certain well defined external conditions, and depends upon their occurrence for its validity. In this respect the response of the ventricle to increased load of pressure is different from that to increased filling, since in the latter case it is the single characteristic of stretching under stress that determines the heart's

* Aided by a grant from the National Research Council.

response. This is an intrinsic property of the heart muscle itself, whereas in the case of the response to increased pressure, as will be seen from the data presented, the response is a function of extrinsic factors.

METHODS. The isolated ventricle of the common sand turtle of from six to twelve inches size has been employed. The ventricle C was fastened by a ligature around the atrioventricular ring onto a cannula whose orifice extended through the A-V valves. One arm of the cannula, as indicated in figure 1, connects through a valve with the venous reservoir A, whose height is adjustable. The other arm communicates with a properly sensitive membrane manometer and, after passing a valve and a screw clamp pressure regulator F, empties into the venous reservoir. The characteristics

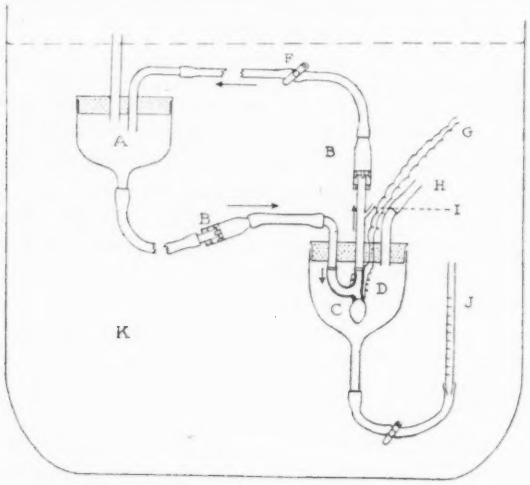

Fig. 1

of the valves are important, as it is necessary to have free forward flow at low pressures but no back leakage. Those used here follow the design described by Decherd and Visscher (1933). Ringer's fluid with one-fourth its volume of the animal's blood was used for perfusion. The heart was suspended in a closed chamber serving as a cardiometer communicating with a recording tambour. The heart was stimulated through platinum electrodes G caught in the tissue above the A-V ligature with break induction shocks delivered at the desired rate by a variable speed make shock eliminator in conjunction with a Harvard inductorium. The shocks were spaced at several rates above the spontaneous rhythm of the isolated ventricle. The temperature was maintained by immersing the system in a water bath of 10 liters capacity adjusted to the proper temperature. The

arterial pressure with which we are concerned is the maximum pressure reached during the ejection period and the venous pressure is measured as the level in centimeters of the fluid in the venous reservoir above the center of the heart.

The experiments were performed by measuring the diastolic volume and the output at several arterial pressures for a constant venous pressure, heart rate and temperature. This was repeated for a range of venous pressures at a given temperature and heart rate. The whole procedure

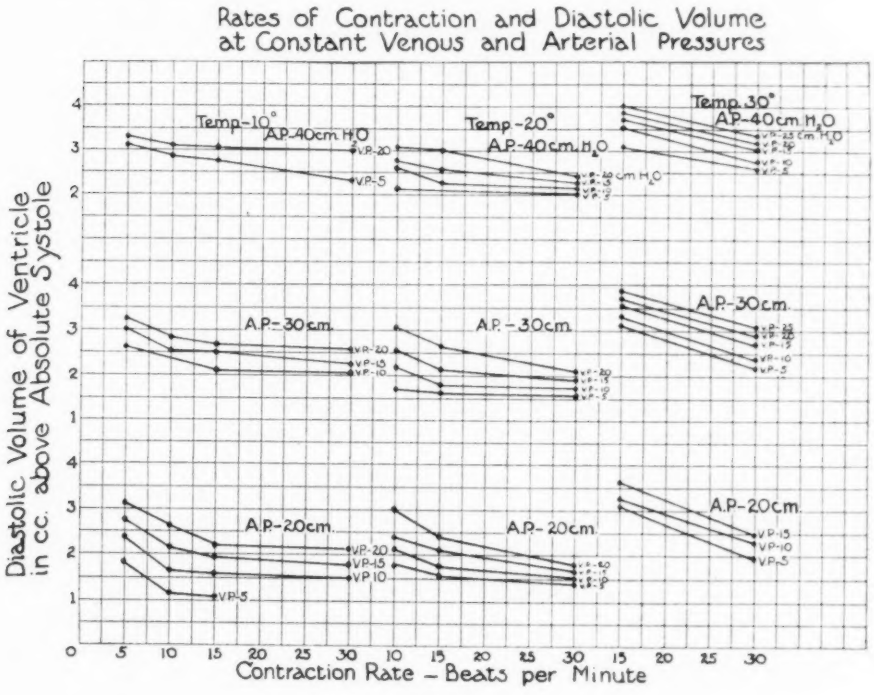

Fig. 2a

was then repeated for the several heart rates employed and the three temperatures used. At least 90 separate observations were thus made in each complete experiment. Part or all of the range of observations reported was made on 40 hearts.

EXPERIMENTAL RESULTS. In order to quantitatively describe the influence of all of the important variables upon the diastolic volume of the ventricle, it is necessary to consider the influence of each controlling factor studied separately while the other factors are held constant. The varia-

bles concerned are 1, the filling or venous pressure; 2, the emptying or arterial pressure; 3, the rate of contraction, which controls the time for filling, and 4, the temperature, which determines the rate of relaxation, the energy liberation in, and the duration of the contraction period, as well as certain physical characteristics of the system itself, such as the coefficient of viscosity of the fluid. The essential data of a single experiment with respect to these variables can be plotted in a family of curves

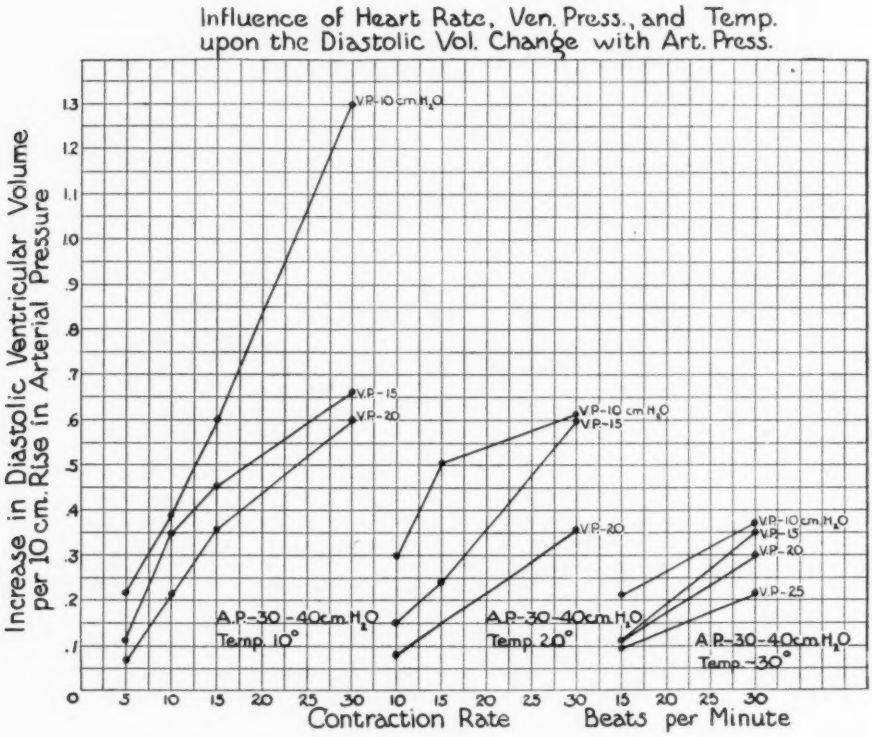

Fig. 2b

relating the diastolic volume of the ventricle to the contraction rate at the several arterial and venous pressures and temperatures. The data of a single experiment are thus presented in figure 2a.

A number of familiar as well as some previously unobserved facts are apparent when such an experiment is studied. It is seen, first of all, that the diastolic volume, at constant arterial pressure, heart rate, and temperature is directly proportional to the filling pressure. This is apparent by

comparing corresponding points on the curves relating venous pressure to diastolic volume at any particular rate. At a given rate the volume always increases with an increase in filling pressure. Furthermore it is seen that with increasing contraction rate, other conditions being held constant, the diastolic volume diminishes. This is evident from the fact that the slope of all the curves is negative. These are well known phenomena and have been thoroughly studied by Starling (1915), Scheinfinkel (1931) and others.

It can also be observed, however, that the change in diastolic volume with alterations in the emptying or arterial pressure depends largely upon the contraction rate and the filling pressure. These relations, which have not been previously described, are more easily seen in figure 2b, where ΔV, the increase in diastolic volume produced by increasing arterial pressure from 30 to 40 cm. H_2O under otherwise constant conditions, is plotted for various values of venous pressure, contraction rate and temperature. ΔV is seen to be nearly zero at low rates at all temperatures and all venous pressures. The value rises with the rate, more strongly with low than high pressures. The value of ΔV is inversely proportional to the venous pressure at all contraction rates and at any temperature.

The dependence of the $\frac{\Delta V}{\Delta P}$ upon the filling pressure is due to the fact that if time is allowed between contractions for the filling curve to reach its plateau for a given venous pressure, the extent to which the ventricle is emptied during contraction will have no influence upon the volume reached during diastole. Consequently if the filling pressure is relatively high the value of $\frac{\Delta V}{\Delta P}$ will approach zero. If the filling pressure, on the other hand, is so low that the contractions fall in the period of rising ventricular volume during filling, the extent of systolic emptying does have an appreciable effect upon the diastolic volume.

The effect upon $\frac{\Delta V}{\Delta P}$ of alterations in the rate of contraction, with other conditions held constant, is also dependent upon the slope of the filling curve at the onset of systole with the various rates. At the lower rates the filling curve has reached a plateau or diastasis, in Henderson's terminology, before the onset of each succeeding contraction. There is consequently little or no difference in the diastolic volume reached due to differences in the systolic volume incident to changes in the extent of ejection, at the lower rates. The value of $\frac{\Delta V}{\Delta P}$ is thus directly proportional to the contraction rate.

The effect of changes in temperature upon the response of the ventricle to alterations in its loading is a complicated one. It has been found quite consistently that as between 10° and 30°C. the diastolic volume of the

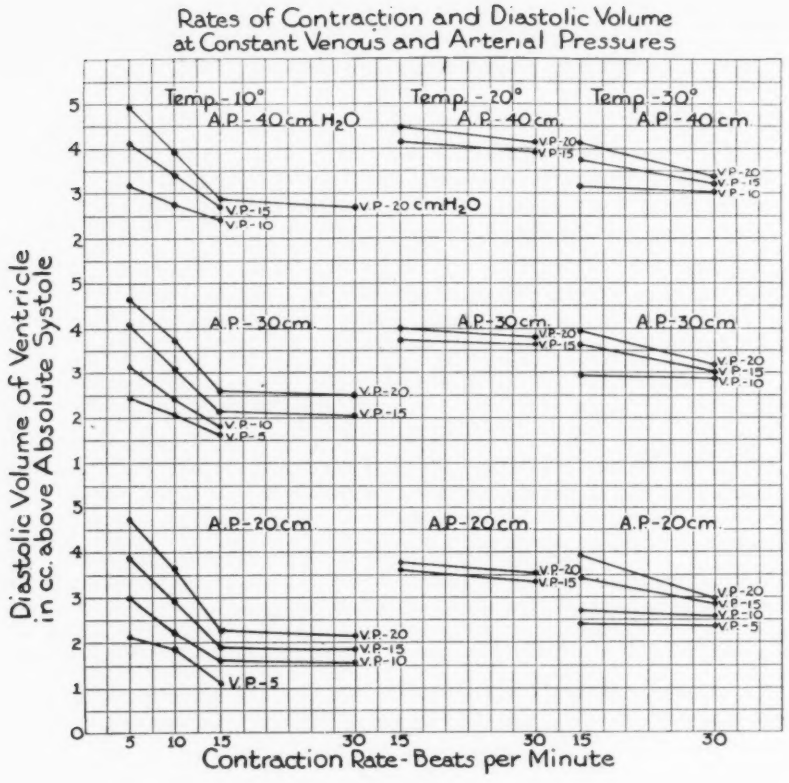
Rates of Contraction and Diastolic Volume
at Constant Venous and Arterial Pressures
Temp.-10°
A.P.-40 cm. H2O
Temp.-20°
A.P.-40 cm.
Temp.-30°
A.P.-40 cm.
V.P.-20 cm H2O
V.P.-15
V.P.-10
A.P.-30 cm.
V.P.-20
V.P.-15
V.P.-10
V.P.-5
A.P.-20 cm.
Diastolic Volume of Ventricle
in cc. above Absolute Systole
Contraction Rate-Beats per Minute

Fig. 3a

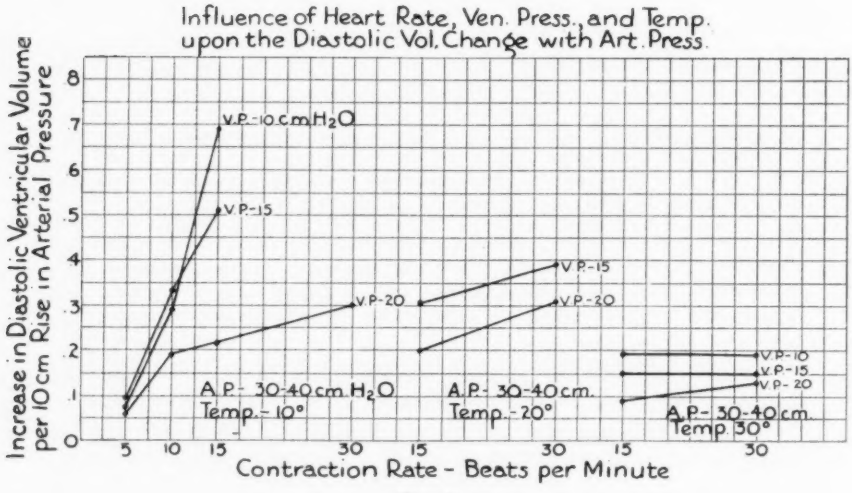
Influence of Heart Rate, Ven. Press., and Temp.
upon the Diastolic Vol. Change with Art. Press.
V.P.-10 cm H2O
V.P.-15
V.P.-20
A.P.-30-40 cm H2O
Temp.-10°
A.P.-30-40 cm.
Temp.-20°
A.P.-30-40 cm.
Temp. 30°
Increase in Diastolic Ventricular Volume
per 10 cm Rise in Arterial Pressure
Contraction Rate - Beats per Minute

Fig. 3b

ventricle at a given arterial pressure, venous pressure and rate is greater at the higher temperature. Between these points, however, different experiments have shown no uniformity in response. That is to say, at 20° the diastolic volume may be either greater than, equal to or less than the diastolic volume at the higher and lower temperatures. In the case of the experiment shown in figure 2 the diastolic volume at the contraction rate of 30 per minute is found to be less at 20° than at either 10° or 30°, under any particular conditions of loading. In the experiment shown in figure 3, however, it will be noted that at 20°C. the diastolic volume at the heart rate of 30 per minute is higher for any particular conditions of loading than at either 10° or 30°. This variability we are inclined to interpret as being due to the unequal opposing interplay of two sets of factors controlling the diastolic ventricular volume. These factors are the extent of systolic emptying, depending on the energy liberation, on the one hand, and the filling rate on the other. It was pointed out by Smith and Visscher (1930, 1931) that the energy liberation in a single contraction of striated muscle as measured by the lactic acid production is inversely proportional to the temperature, but that the rate of anaerobic recovery is directly proportional to the temperature and that consequently in a rapid series of contractions, as was also pointed out by Hartree and Hill (1921) on the basis of heat production studies, the energy liberation per contraction increases with the temperature. If the energy liberation in contraction is high, one would expect the diastolic ventricular volume to be relatively low, particularly at the higher heart rates, because the systolic emptying will be greater the more energy is liberated in contraction. The temperature also affects the rate of relaxation of the muscle wall, which plays a very great rôle in controlling the rate of filling at a given venous pressure. The temperature likewise controls the coefficient of elasticity of the resting muscle. It has not been possible in these studies to untangle the part played by each of these several factors when the temperature is altered. It is only possible at the present time to say that these factors are operative and that they must be completely taken into account if the influence of temperature upon the level of diastolic volume is to be thoroughly understood.

The effect of temperature changes upon $\frac{\Delta V}{\Delta P}$, the change in diastolic volume with variations in emptying pressure, is more uniform. We have found in general, as figures 2b and 3b and table 1 show, that the increment in diastolic volume with rise in arterial pressure becomes smaller at the higher temperatures at all filling pressures and all rates. This is true regardless of the absolute level of the diastolic volume at a given temperature, as is seen by the fact that the relationship is the same whether the volume at 20° was lower or higher than at 10° and 30°, as shown in the experiments respectively in figures 2 and 3. In a number of experiments

we have found that the increment in diastolic volume with rise in arterial pressure is actually zero at the low heart rates and particularly at the higher temperatures. This is well illustrated in the experiment shown in table 1. In this instance the mechanism for increase in energy liberation by increasing the diastolic volume, with increasing arterial pressure, is seen to be entirely absent at 10° at the rates of 5 and 10 beats per minute at the highest filling pressure used. At 20° ΔV is zero even at 15 beats per minute, and at 30° at 30 beats per minute at the same venous pressure. It is therefore apparent that at the higher venous pressures at the rates

TABLE 1

Influence of heart rate on diastolic volume change with arterial pressure

TEMPERATURE	VENOUS PRESSURE CM. H_2O	ΔV: INCREASE IN DIASTOLIC VOLUME IN CC. FOR INCREMENT IN ARTERIAL PRESSURE FROM 30 TO 40 CM. H_2O			
		Beats per minute			
		5	10	15	30
10°	5	0.05	0.38	0.55	0.60
	10	0.00	0.32	0.58	0.60
	15	0.00	0.28	0.52	0.60
	20	0.00	0.20	0.30	0.52
	25	0.00	0.00	0.10	0.15
20°	5		0.00	0.04	0.53
	10		0.00	0.00	0.37
	15		0.00	0.00	0.20
	20		0.00	0.00	0.10
	25		0.00	0.00	0.05
30°	5			0.12	0.22
	10			0.07	0.09
	15			0.06	0.05
	20			0.00	0.00
	25			0.00	0.00

indicated the arterial pressure is entirely without effect upon the diastolic volume and therefore, as Starling and Visscher (1927) have shown, upon the energy liberation. Stella (1931) believed that he had observed an increased energy liberation with increasing arterial pressure at constant diastolic volume, in contradiction to the results of Starling and Visscher, Clark and White (1930), Eismayer and Quincke (1930) and others. Decherd and Visscher (1933) have been unable to confirm Stella in his observation that the energy liberation varies in proportion to the work done by the turtle's ventricle.

Krogh (1912) investigated in an indirect way the influence of venous

pressure upon the output of the intact mammalian heart. He found that with higher filling pressures the stroke output remained relatively constant with varying heart rates. It could probably be deduced from his observations that under conditions of high filling pressure the diastolic volume of the heart is less dependent upon the rate of contraction than at the lower filling pressure. The observations recorded here give experimental demonstration that such a deduction would be correct.

It becomes apparent therefore from these experiments that the response of the ventricle to change in pressure is a function of external rather than internal conditions. Whether or not there will be an increase in diastolic volume with increased arterial pressure depends upon the point on the filling curve after systole that a new contraction begins. If the filling curve has reached its plateau, as occurs at the lower heart rates, the higher venous pressures and the higher temperatures, the arterial pressure is without effect upon the diastolic volume because the extent of emptying has no influence upon the level of filling reached in diastole. The so-called "law of the heart" with respect to the adaptation to increased pressure load must therefore be modified to include the statement that the heart's mechanism for automatic adaptability to increased pressure depends upon these factors just enumerated, and is not operative at all under certain conditions.

CONCLUSIONS

1. The increase in diastolic volume of the ventricle with increased arterial pressure depends upon the contraction rate, the filling pressure and the temperature. The magnitude of $\frac{\Delta V}{\Delta P}$ varies directly with the contraction rate, and inversely with the other two variables.

2. Below a certain critical contraction rate and above a critical filling pressure the diastolic volume is independent of the arterial pressure, within the ranges studied. This relation is to be expected because with low rates and high venous pressures the extent of diastolic filling is not so much influenced by the extent of systolic emptying as it is at higher rates and low filling pressures.

3. The "law of the heart" with respect to the adaptation to increased pressure is not an expression of an intrinsic property of the cardiac muscle but depends upon the existence of proper extrinsic conditions regarding rate, temperature and filling pressure.

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LACTIC ACID FORMATION IN MEDULLATED NERVE

FRANCIS O. SCHMITT AND CARL F. CORI

From the Departments of Zoology and Pharmacology, Washington University, Saint Louis

Received for publication July 8, 1933

It has been shown by Meyerhof and Boyland (1931) that the respiratory quotient of frog muscle poisoned with iodoacetate is reduced from the normal level of unity to about 0.75 and that addition of lactate to muscle so poisoned causes an increase in the respiratory quotient and in oxygen consumption. Mawson (1932) showed that muscles poisoned with iodoacetate contract much longer when lactate is added than without addition of lactate. These findings were interpreted to mean that lactate was oxidized in the poisoned muscles and that this oxidation diminished the degree of poisoning. Similarly, Feng (1932) found that lactate serves to protect nerves from the effect of iodoacetate in air but not in nitrogen, which suggests that nerve may be able to oxidize lactic acid. In contrast to these recent observations, Gerard and Meyerhof (1927) and Holmes and Gerard (1929) found that while nerve produced lactic acid under asphyxial conditions, there was no removal of lactic acid upon readmission of oxygen.

According to Gerard and Meyerhof (1927) and Holmes, Gerard and Solomon (1930), stimulation of nerves either in oxygen or in nitrogen does not lead to production of lactic acid. In this connection it was noted that when eight to twelve nerves were laid over electrodes side by side and stimulated, as in the experiments of the authors quoted above, a tenfold increase in the shock strength which would be maximal for a single nerve was still subthreshold for the group of nerves owing to a shunting effect of saline solution. It was necessary to install a 1 mfd. condenser in the Thyratron stimulating device to obtain maximal action potentials at high rates of stimulation, as measured by the cathode ray oscillograph. Obviously experiments designed to show chemical or metabolic effects of stimulation, especially if they necessitate simultaneous stimulation of several nerves laid over the same electrodes, should be very carefully controlled as to the action potentials elicited by the stimulation. It is our impression that this precaution has not been scrupulously enough observed in the past.

In other experiments it was found that it was possible to expose nerves

to mixtures of carbon monoxide and oxygen such that the action potential remained full size indefinitely, although the oxygen consumption was reduced 50 per cent or more. This suggested the possibility that under these conditions removal of lactic acid might be interfered with and hence very suitable conditions be provided for a study of the effect of stimulation on the lactic acid production of nerve.

These facts, together with the recent work mentioned above which indicates that oxidative removal of lactic acid may be possible in nerve under certain conditions, made it desirable to reinvestigate certain phases of lactic acid metabolism in nerve. Since the completion of the experimental work a paper has appeared by Chang and Gerard (1933) in which the work of Feng (1932) is confirmed and extended. This will be discussed later.

METHODS. The nerves were obtained from medium size R. pipiens which were kept in the ranarium at a temperature of about 18°C. for a week or so after shipment. It was found that the rate of formation of lactic acid varied in nerves taken from different shipments of frogs, hence we attempted to make as many determinations as possible on a few large shipments; these were obtained during the months of March and April. The nerves were dissected as rapidly as possible, care being taken to avoid injury by tearing or stretching. On an average the two sciatics from a frog required four minutes for dissection. As a rule, immediately after dissection the partner nerves from each frog were placed on glass plates designated as *A* and *B* and kept in a chilled Petri dish which served as a moist chamber. In some instances the nerves were placed in chilled Ringer solution immediately upon dissection. In all cases, after removing the superficial liquid, the nerves were transferred to glass slides and weighed. Control experiments showed that the weights so obtained were accurate within a few per cent.

In the experiments on resting lactic acid production the nerves, usually ten to twelve in number, were laid lengthwise on glass plates and the plates then inserted into a moist chamber through which the desired gas mixture could be passed. For the experiments on stimulated nerves, special glass plates were made on the surface of each of which two pairs of platinum wires were sealed, one pair for stimulation and the other pair to lead off the action potential to the oscillograph. These platinum wires extended over the sides of the plates and dipped into pools of mercury which connected to the appropriate stimulating and lead-off wires in the hard rubber chamber. In this way no lactic acid which might have diffused out of the nerves during the experiment was lost, for at the end of the experiment the plates with the nerves lying on them were simply lifted from the hard rubber chamber and plunged into the cold fixative.

Carbon monoxide was prepared by dripping concentrated formic acid

into hot concentrated sulphuric acid, the gas being stored in large carboys. The appropriate mixtures of carbon monoxide with oxygen were made and analyzed with the Henderson-Haldane apparatus. For simple asphyxia, the hydrogen was purified by passing it over glowing copper and thence through a train to remove CO_2 and acid and finally through Ringer solution.

The nerves were stimulated by means of the Thyratron device described by Schmitt and Schmitt (1932) and the action potentials measured on the Braun tube.

Lactic acid determinations were carried out as follows. The glass plates containing the nerves were plunged into centrifuge tubes containing 3 cc. of ice-cold $HgCl_2$—HCl solution (2.5 per cent $HgCl_2$ in 0.5 N HCl). In this manner the nerves were submerged at once in the fixative solution and fluid adhering to the outside of the nerves and to the glass plate was included in the analysis. Sometimes the analyses were performed on the same day, while in other cases the sample was kept over night in the ice-box. Precipitation of proteins with $HgCl_2$—HCl (Schenk, 1893) has been used repeatedly for lactic acid determinations in tissues and has been found not to cause any loss of lactic acid (Cori, 1925).

The contents of each tube were transferred to a mortar, about 0.5 gram of acid-washed quartz sand added, and the whole mass ground very thoroughly. Twenty cubic centimeters of water were then added, part of which was used to rinse off the glass plate on which the nerves were kept. The total volume was thus 23 cc. plus the water content of the nerves, which was assumed to be 80 per cent. After thorough mixing, the whole mass was centrifuged and the supernatant fluid poured off and freed of Hg in the usual manner. The mercury-free solution was treated with solid $CuSO_4$ and $Ca(OH)_2$. Although this procedure has become routine in lactic acid determinations, it was omitted in a number of cases because it was found that it made no difference in the values obtained. With care it was possible to obtain 20 cc. for the lactic acid analysis, which corresponds to 86 per cent of the sample.

For the lactic acid determination a micro-adaptation of the Friedemann-Cotonio-Shaffer (1927) method was used. The principal features of this micro-determination, as developed by Wendel,[1] are an all glass distillation apparatus, the elimination of practically all reagent-blank (less than 0.02 cc. of 0.005 N I_2), a small final volume for the starch-iodine end-point (about 10 cc.) and the possibility of determining as little as 0.05 mgm. of lactic acid with a fair degree of accuracy. In order to keep the blank as small as possible, several of the chemicals used, especially the $CuSO_4$ and

[1] We wish to express our appreciation to Doctor Wendel for supplying us with information concerning this unpublished technique. The details will be described by him elsewhere.

the $Ca(OH)_2$ were recrystallized or purified. Sulphur-free rubber tubing was used for all connections and rubber stoppers were boiled very thoroughly in alkali and acid. The air entering the distillation apparatus was filtered and washed. Filter paper was washed in water and dried before being used.

As may be seen in the tables the amount of lactic acid determined was generally between 0.1 and 0.2 mgm.; in only a few cases was it between 0.07 and 0.1 mgm., which is still well within the allowable range of this method. Experiments with known solutions of Zn-lactate showed a recovery of 97 to 99 per cent for amounts of lactate corresponding to those encountered in the present work. The following example is typical as an illustration of the reproducibility of results. Four consecutive analyses of a sample containing 0.15 mgm. of lactic acid gave the following titrations in terms of 0.005 N I_2; 0.65, 0.64, 0.66, 0.65 average, 0.65 cc. or 0.146 mgm. of lactic acid.

RESULTS. *1. Lactic acid in resting nerves.* Gerard and Meyerhof (1927) reported six experiments on esculenta and temporaria nerves dissected at 0°. The values varied between 70 and 160, with an average of 107 mgm. per cent. Holmes, Gerard and Solomon (1930) obtained somewhat lower values in 17 experiments on bull-frog nerves, namely, an average of 80.7 mgm. per cent, when corrected for the loss of lactic acid inherent in their method. Here again the nerves were chilled prior to analysis. According to these results resting frog nerve would contain a much higher amount of lactic acid than resting frog muscle.

In three experiments in table 1 (nos. 1, 6a, 10a) nerves were dissected as described earlier in the text and kept in a chilled moist chamber until a sufficient number of nerves had been collected. They were then fixed all at one time in the ice-cold extraction fluid. Values of 38, 31 and 30 mgm. per cent were found. In two experiments (nos. 2a, 3a) the nerves were kept in chilled Ringer solution before being fixed in the $HgCl_2$–HCl solution. The values were somewhat lower (24 and 25 mgm. per cent, respectively), perhaps owing to diffusion of lactic acid into the Ringer solution. Partial drying of nerves during the 30 to 45 minutes required for dissection, which happened inadvertently in two experiments (nos. 13a, 13b), resulted in a marked increase in lactic acid content.

It is difficult to say why previous investigators obtained resting lactic acid values for nerve, which are two to three times as high as those here reported. Apparently nerves are easily damaged and respond under such circumstances with an increased production of lactic acid; the effect of partial drying is a case in point. At any rate, the low resting lactic acid values found in the present work should afford a better base-line for experimental procedures than the high and often irregular values reported by previous workers.

TABLE 1

Lactic acid production of resting nerves under various conditions

EXPERIMENT NUMBER	WEIGHT OF NERVE	LACTIC ACID				PROCEDURE
		Mgm.	Mgm. per cent	Formed per hour	Removed per hour	
	mgm.					
1	453	0.172	38			Immediately after dissection
2* a	349	0.084	24			a. Immediately after dissection
b	353	0.208	59	10.8		b. In H_2 for 195 min. Act. Pot. gone in 160 min.
3* a	316	0.079	25			a. Immediately after dissection
b	336	0.232	69	16.0		b. In H_2 for 165 min. Act. Pot. gone in 100 min.
4 a	311	0.295	95	27.5		a. In H_2 for 135 min.
b	325	0.260	80		7.5	b. Same. Act. Pot. gone in 115 min. Then O_2 for 120 min. Act. Pot. normal 15 min. after readmission of O_2
5 a	317	0.266	84	24.5		a. In H_2 for 125 min.
b	336	0.215	64		3.8	b. Same. Act. Pot. gone in 105 min. Then in air for 317 min. Act. Pot. normal 15 min. after readmission of air
6 a	359	0.111	31			a. Immediately after dissection
b	349	0.450	129	28		b. In CO with 1.5% O_2 for 210 min. Act. Pot. gone in 200 min.
7 a	326	0.587	180	25.5		a. In CO with 6.4% O_2 for 345 min.
b	322	0.457	142		7.9	b. Same. Act. Pot. normal. Then in O_2 for 290 min.
8 a	300	0.168	56			a. In air in Warburg vessel for 280 min. O_2 cons. = 51.5 cu. mm./gr./hr.
b	297	0.306	103	10		b. In CO with 6.4% O_2 in Warburg vessel for 280 min. O_2 cons. = 21.5 cu. mm./gr./hr. Inhibition = 58%
9 a	304	0.207	68			a. In air in Warburg vessel for 400 min. O_2 cons. = 66 cu. mm./gr./hr.
b	291	0.183	63			b. In CO with 16.1% O_2 in Warburg vessel for 400 min. O_2 cons. = 68 cu. mm./gr./hr. No effect on respiration

TABLE 1—*Concluded*

EXPERIMENT NUMBER	WEIGHT OF NERVES	LACTIC ACID				PROCEDURE
		Mgm.	Mgm. per cent	Formed per hour	Removed per hour	
	mgm.					
10 a	279	0.083	30			a. Immediately after dissection
b	269	0.078	29			b. Kept on glass plate in air for 360 min.
11 a	277	0.196	71	15.2		a. In H_2 for 150 min.
b	280	0.129	46		5.4	b. Same. Then in O_2 for 280 min.
13 a	220	0.114	52			a and b nerves partially dried during dissection.
b	184	0.081	44			
14 a	236	0.248	105	20.1		a. In H_2 for 215 min.
b	268	0.241	90		4.5	b. Same. Then in O_2 for 200 min.

* The nerves in these experiments were kept in chilled Ringer solution during dissection preliminary to weighing them and placing them in H_2. In all other experiments the nerves were preserved in chilled moist chamber preparatory to weighing.

2. Effect of anaerobiosis in H_2 and CO. The effect of anaerobiosis on the rate of lactic acid production in nerve was investigated by Gerard and Meyerhof (1927) by chemical and manometric methods. They found that the rate increased up to the second hour and diminished after the fourth hour. The decrease in the rate of lactic acid formation was shown to be due to lack of carbohydrate, for when nerves were suspended in Ringer solution containing glucose, a uniform rate was maintained for more than 30 hours. Although symmetrical nerves gave values which agreed generally within 10 per cent, considerable variations were encountered in individual experiments. This was attributed to factors which have an influence on the carbohydrate reserve of the nerves, such as seasonal variation, temperature and state of nutrition of the frogs. Holmes and Gerard (1929) showed that there was a close agreement between loss of carbohydrate and increase in lactic acid of rabbit nerve kept at rest in nitrogen.

The following values were reported by Gerard and Meyerhof (1927). At 15° the maximal hourly rate of lactic acid formation was 7.4 without and 12.5 mgm. per cent with addition of glucose. At 28° in a glucose-Ringer solution the rate was 25 to 30 mgm. per cent. Results obtained in the present experiments agree fairly well with these figures, if allowance is made for the effect of temperature. The nerves were kept at about 22° and showed an average hourly rate of lactic acid formation in H_2 of 20.6,

with variations between 15.2 and 27.5 mgm. per 100 grams nerve per hour. Experiment 2b, which showed a low rate of lactic acid formation, is not included in the above average, because the nerves were kept on a moist filter paper; this doubtless allowed some diffusion of lactic acid to take place. In all other experiments the nerves were kept on glass-plates as described earlier in the text. In a control experiment (no. 10b) in which the nerves on the glass plate were kept in air instead of hydrogen, there was no change in the lactic acid content.

In CO with 1.5 to 4.5 per cent O_2 (expt. 6b, 18a) lactic acid formation proceeded at a rate of 28 and 17.7 mgm. per cent, i.e., the rate was about the same as in pure hydrogen. The same result was obtained in two experiments (nos. 7a, 15a) with CO containing 6 per cent O_2. In four other experiments (nos. 8b, 12a, 16a, 17a) with the same gas mixture, the hourly rate was somewhat lower than in pure hydrogen, being only 7.1 to 11.4 mgm. per cent. Apparently, CO containing 6 per cent O_2 is just on the borderline as regards maximal rate of lactic acid formation in nerve, although respiration was found to be inhibited only 50 to 70 per cent. In one experiment (no. 9b) the nerves were kept in CO containing 16 per cent O_2 and as a control the symmetrical nerves were kept in air. No significant difference in the lactic acid content could be detected, so that this particular CO concentration is definitely non-glycolytic for nerve. There was no inhibition in O_2 consumption at this CO concentration. Incidentally it was noted in this and in other experiments that shaking nerves for several hours in vessels attached to Warburg manometers has a somewhat damaging effect on nerves, as shown by an increase in the lactic acid content above the resting value. Nerves kept in air without shaking do not show this increase in lactic acid content.

3. Removal of lactic acid in O_2. The preceding experiments show that inhibition of nerve respiration by means of CO or H_2 causes an accumulation of lactic acid. From this it may be inferred that the normal respiratory mechanism of nerve prevents the appearance of lactic acid, but it is not clear whether this is due to the fact that lactic acid fails to be formed under these conditions, or whether lactic acid is removed as fast as it is formed. Gerard and Meyerhof (1927) favored the former possibility. They published one experiment in which 3 groups of nerves were first kept for 17 hours in nitrogen. Two groups of nerves analyzed at the end of this period showed 110 and 140 mgm. per cent lactic acid. Following the period of anoxia the third group of nerves was kept for 4 hours in oxygen and showed 130 mgm. per cent lactic acid. Holmes and Gerard (1929) performed similar experiments on rabbit nerve with uniformly negative results, i.e., there was no disappearance of lactic acid in oxygen. On the other hand, Gerard and Meyerhof (1927) reported two experiments in which nerves were kept in a Ringer solution containing lactate. Re-

tention of CO_2 was determined manometrically and served as a measure of the disappearance of lactate. It was found that the amount of lactate which disappeared, assuming that it was oxidized, could have accounted for about 45 per cent of the total O_2 consumption of nerve. Quite recently Chang and Gerard (1933), on the basis of experiments with nerve poisoned with iodoacetate, came to the conclusion that nerve may be able to oxidize lactic acid. They found, in extension of earlier experiments of

TABLE 2

The effect of stimulation on the lactic acid production of nerve in carbon monoxide

EXPERIMENT NUMBER	WEIGHT OF NERVES	COMPOSITION OF GAS	PERIOD OF ASPHYXIA	RATE OF STIMULATION	MAXIMUM ACTION POTENTIAL HEIGHT IN PER CENT OF NORMAL	LACTIC ACID		
						Mgm.	Mgm. per cent	Formed per hour
			minutes	*shocks per second*				
12	a 296	CO = 94%	180		60	0.186	65	10.7
	b 290	O_2 = 6%	180	200	15	0.174	60	
15	a 289	CO = 94%	68		45	0.188	65	28.2
	b 286	O_2 = 6%	68	425	0	0.166	58	
16	a 360	CO = 94%	305		25	0.221	69	7.1
	b 320	O_2 = 6%	305	68	425	0		
				22	125	5		
				215	35	17	0.211	66
17	a 265	CO = 94%	375		100	0.275	104	11.4
	b 265	O_2 = 6%	375	117	425	4		
				91	125	12		
				167	35	40	0.273	103
18	a 287	CO = 94%	210		89	0.273	95	17.7
	b 289	O_2 = 6 %	210	71	425	5		
				55	125	0		
				31	35	13		
				53	10	13	0.309	107

Feng (1932) that addition of lactate protected nerves against iodoacetate poisoning, both as regards conduction and O_2 consumption. Thus, in M/15 iodoacetate nerve respiration and conduction were abolished in the second hour, but when M/30 lactate was added, one-third of the O_2 consumption was still present after 7 hours and conduction was maintained for the same period of time.

In five experiments (nos. 4, 5, 7, 11, 14) reported in table 1 a decrease in lactic acid occurred in each case when nerves previously asphyxiated in

H_2 or CO were allowed to recover in oxygen. The period of anoxia lasted only 2 to 6 hours, as compared with 17 hours in the experiments of Gerard and Meyerhof (1927). Two to 5 hours were allowed for recovery in oxygen; this resulted in a decrease in lactic acid amounting to 15 to 38 mgm. per cent. That this is a significant decrease is shown by the fact that in five experiments (nos. 12, 15, 16, 17, 18, table 2), differences of only 5, 7, 3, 1 and 12 mgm. per cent lactic acid were observed, when two sets of symmetrical nerves were analyzed immediately after a period of asphyxia.

The rate of disappearance of lactic acid in the above experiments varied between 3.8 and 7.9, the average being 5.8 mgm. per 100 grams per hour. This would require an O_2 consumption of 4.3 cc. per 100 grams per hour for complete oxidation. Since nerves recovering in O_2 after a period of asphyxia were found to consume about 6.0 cc. per 100 grams per hour at 22°, their O_2 consumption would be more than sufficient to account for the observed rate of disappearance of lactic acid. However, if only the extra O_2 consumption of previously asphyxiated nerve is taken into consideration, more lactic acid would disappear than could be removed by oxidation.

The same nerves under anaerobic conditions produced lactic acid at an average rate of 22.5 mgm. per 100 grams per hour, which is about 4 times as fast as the nerves are able to remove lactic acid in oxygen. Yet, when resting nerves are kept in oxygen, no accumulation of lactic acid takes place. If one assumes that oxidation is the only way in which nerve can dispose of lactic acid, the rate of its production must be considerably smaller under aerobic than under anaerobic conditions. A second possibility is that nerve, in analogy to muscle, is capable of reconverting part of its lactic acid to carbohydrate. In this case the rate of lactic acid production could be the same under aerobic and anaerobic conditions. Carbohydrate analyses before and after recovery of nerve in oxygen are required to decide between these two possibilities.

4. Effect of stimulation. Gerard and Meyerhof (1927) failed to find an effect of stimulation on the rate of lactic acid production of nerve kept under anaerobic conditions. Negative results were also reported by Holmes, Gerard and Solomon (1930) who determined free sugar and glycogen in addition to lactic acid. The present experiments (nos. 12, 15, 16, 17, 18, table 2) confirm the results of the above investigators. Use was made of the fact that CO containing 6 per cent O_2, while allowing lactic acid to accumulate in nerve has no effect on the action potentials at very low rates of stimulation and does not abolish them unless the rate of stimulation is very high. In previous investigations the nerves were kept in pure nitrogen and, as is well known, nerve conduction is quickly abolished under these conditions. The period of stimulation varied between 1 and 6 hours. The customary procedure was to stimulate one group of nerves

very rapidly (425/sec.) until the action potential was greatly decreased and tended to show alternation of response to stimuli. At this time the rate was decreased so that the action potential was again high. This procedure was followed until the end of the period and resulted in maintaining the nerve in a maximal state of activity. Figure 1 is illustrative of the data obtained in these experiments.

In only one out of five experiments was the difference in lactic acid content between the stimulated nerves and their resting controls greater than

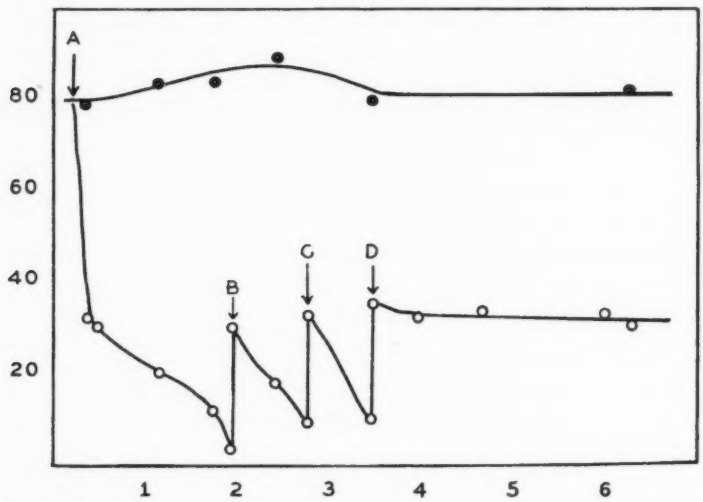

Fig. 1. Effect of stimulation on action potential of nerves poisoned by CO containing 6 per cent O_2. Ordinates, millimeter spike height measured on the Braun tube; abscissae, time in hours. The CO was passed into both nerve chambers at *A*. The control nerve (closed circles) was tested only occasionally while the experimental nerve (open circles) was stimulated continuously. At *A*, the rate was 425 per second, at *B* the rate was decreased to 125 per second, at *C* the direction of polarization was reversed, at *D* the rate was decreased to 35 per second.

10 mgm. per cent. In the other cases the difference ranged between 1 and 7 mgm. per cent, clearly a negative result which may also serve as an index of the error of the methods used.

SUMMARY

1. Frog nerve immediately after dissection or after being kept for some hours in a moist chamber in air was found to contain about 33 mgm. per cent lactic acid, in contrast to values of 70 to 160 mgm. per cent reported by others.

2. In pure H_2 at 22° nerve forms lactic acid at a rate of about 20 mgm.

per cent per hour. In a gas mixture containing 94 per cent CO and 6 per cent O_2 lactic acid formation may take place as rapidly as in pure H_2 although the respiration under these conditions is decreased but 50 to 70 per cent and the irritability is affected only slightly.

3. Lactic acid which has accumulated in H_2 or in CO may be partially removed upon readmission of O_2. This removal averages about 6 mgm. per cent per hour.

4. In the $CO:O_2$ mixture, stimulation at high rates and at intensities maximal for A spikes as recorded oscillographically calls forth the production of no excess lactic acid in the stimulated nerve as compared with the unstimulated partner nerve in the same gas mixture.

The expenses of this investigation were defrayed by a Research Grant to Washington University by the Rockefeller Foundation.

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STUDIES ON FLUORINE IN THE NUTRITION OF THE RAT[1]

I. Its Influence upon Growth

ALVIN R. LAMB, PAUL H. PHILLIPS, E. B. HART AND G. BOHSTEDT

From the Department of Agricultural Chemistry, and the Department of Animal Husbandry, University of Wisconsin, Madison

Received for publication July 14, 1933

Interest in the matter of the toxicity of small amounts of fluorine in the ingesta of man and animals, as well as in the possible rôle of this element in nutrition, has much increased in recent years. The discovery of the association of fluorine in certain water supplies with mottled enamel in teeth (2, 6, 7, 9), the use of insecticides whose active principle is the fluorine ion, and possible toxic effects from the use of rock phosphate containing fluorine as a fertilizer (1) and as a constituent of animal feeds, have made chronic fluorine poisoning a matter of considerable importance.

Comprehensive studies, extending over a five-year period, have been completed on the effects of fluorine upon growth, reproduction, histopathology and the composition of blood and bone. Four species of animals have been used, viz., rats, swine, poultry, and cattle. Much of the data will be published elsewhere, the present report including only the results obtained with rats.

Experimental evidence has been presented from several laboratories to show that fluorine interferes with growth. Sollmann, Schettler, and Wetzel (12) fed various levels of sodium fluoride and rock phosphate. Levels below 0.01 per cent sodium fluoride caused no change in the growth curve. Levels from 0.02 to 0.04 per cent showed a moderate but definite interference with growth while in concentrations above 0.05 per cent a marked retardation in growth occurred. They also found that rock phosphate inhibited growth in a manner similar to sodium fluoride, but that the fluorine in rock phosphate required to produce an equivalent effect was from 2 to 3 times as high as when the more soluble sodium fluoride was used. Schulz and Lamb (8) reported normal growth when 0.05 per cent sodium fluoride was fed to rats, but higher levels, up to 0.25 per cent, caused progressive impairment of growth. The highest level was found to have

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These studies were supported in part through a fellowship granted by the Ruhm Phosphate and Chemical Company of Mt. Pleasant, Tennessee.

very rapid toxic effects, causing death in a few weeks. Kick (3) concluded that 0.05 per cent NaF caused a decrease in the growth of rats. Smyth and Smyth (11) in a study of the relative toxicity of some fluorine and arsenical insecticides reported cryolite and barium fluosilicate to be harmful to growth of rats above a level of 20 to 25 mgm. per kilo of body weight per day. McClure and Mitchell (5) found that fluorine either as calcium or sodium fluoride inhibited growth, entirely aside from any effect of food consumption, when fed at a level of 0.0313 per cent or more. Unpublished data from this laboratory with relatively high levels have shown a high degree of toxicity. One-tenth of one per cent of sodium fluoride or 32 mgm. of fluorine per kilo of body weight per day inhibits growth very sharply. Young rats weighing between 50 and 60 grams each require approximately 6 to 7 weeks to double their weight when fed this level of fluorine, while control rats of the same age will double their weight in approximately three weeks. A level of 0.15 per cent sodium fluoride will allow a very slow gain in weight while 0.20 per cent sodium fluoride, or 72 mgm. of fluorine per kilo of body weight per day will barely allow maintenance of weight over a seven-weeks period. The highest level, or 0.3 per cent sodium fluoride (75 mgm. fluorine per kilo of body weight per day) is sufficiently toxic to cause loss of weight and death in a few weeks. On these higher levels food consumption is greatly reduced in all cases.

Experimental. The basal diet used throughout these experiments was composed of yellow corn 55.75 per cent, wheat middlings 24.0 per cent, linseed oil meal 12.0 per cent, alfalfa meal 3.0 per cent, meat meal tankage 2.0 per cent, special steamed bone meal 1.75 per cent, ground limestone 0.5 per cent, iodized salt 0.5 per cent, and 1 per cent of cod liver oil. This ration was designed to be complete in all of the known dietary requirements for the rat. It allowed for the substitution of varying amounts of rock phosphate or the addition of fluorides without appreciable change in the proportions of its dietary constituents.

The washed rock phosphate used in these studies was from the Tennessee brown rock phosphate deposits and was supplied by the Ruhm Phosphate and Chemical Company of Mt. Pleasant, Tennessee. This material contained approximately 3.5 per cent fluorine. The rock phosphate was substituted at three levels for a similar quantity of the bone meal of the basal ration, and one level each of sodium fluoride and calcium fluoride was likewise used. The experimental groups included the following:

Lots A-1, A-2—Controls.
Lot A-3 Basal ration plus 0.043 per cent NaF
Lot A-4 Basal ration plus 0.040 per cent CaF_2
Lot A-6 Basal ration plus 0.6 per cent rock phosphate
Lot A-7 Basal ration plus 1.0 per cent rock phosphate
Lot A-8 Basal ration plus 2.0 per cent rock phosphate

In addition to the effect on growth, the reproductive function was also studied in these animals except lots A-4 and A-8 which were discontinued. The plan in these studies was to allow the female rats to raise 3 or more litters and then to choose representative young from the last litter and allow them to undergo a like period of reproduction and so on until five generations had been observed. All of the young were kept at least until four to six weeks of age and weighed at weekly intervals during that time. Further, three series of 40 animals each, 20 females and 20 males, were studied on rations A-1, A-3 and A-7 to determine the effect upon oestrum and to study the physiological and histological effects of fluorine upon certain endocrine glands. In this series weekly weights were also taken. Thus these experiments furnish data relative to the birth weights, weaning weights at four weeks of age, mature weights for both males and females, and growth curves for five generations.

Effects on growth. The birth weights obtained indicate that fluorine either as 0.04 per cent sodium fluoride or 1 per cent rock phosphate (0.035 per cent F) may cause a slight reduction in the birth weight of the young. Since all lots were under otherwise identical treatment any variation in weight could be taken as an indication of the effect of fluorine upon birth weight. In some cases the young were not weighed before they had suckled, but these cases were quite uniformly distributed through all the groups, which tends, in a fairly large number of litters, to erase differences due to this factor. The controls, 249 young representing 42 litters from A-1 females, averaged 6.03 grams as against a 5.30 gram average weight for 147 young of 25 litters from A-3 females and a 5.49 gram average for 146 young of 27 litters from A-7 mothers. Although the litter size averaged slightly larger in the A-3 lots, the difference is not sufficiently great to account for the drop below the average birth weight of the control animals.

The weights of the young when weaned at four weeks of age showed much the same general relationship between lots as that described above. Litters were reduced in number to 6 young, and litters below that number are not included in the data. A tabulation of the litters of six raised to weaning age gave the following results: 26 litters from A-1 females averaged 43.4 grams each; 9 litters from A-3 females averaged 34.9 grams each; 11 litters from A-6 females averaged 43.4 grams each; while 13 litters from A-7 females averaged 39.9 grams each. Since relatively little of the doe's ration was eaten by the young up to the 28th day of life it appears that either the vigor of the young or the quality or quantity of the milk was affected by the fluoride in the diet. When the weights of the mature animals were taken as they were removed from the experiments, usually at 12 to 15 months of age, a variation was noted although this might possibly have been due to inherent variations in the original stock; it was interesting

to note that the control males averaged slightly higher in mature weight than the fluorine-fed males. Little or no difference occurred in the mature weights of the females which in this group of rats ranged from 200 to 215 grams.

The growth curves, which are separate averages for the males and females in each lot, are shown in chart 1. It is apparent that fluorine either in the form of sodium fluoride or rock phosphate at the levels fed in these experiments did not appreciably retard growth. If the curves are examined closely it is seen that there is practically no difference in the rate of growth or the average weight attained during the period. It appears that 1.0 per cent or more of rock phosphate (0.035 per cent F) or 0.043 per cent sodium fluoride (0.019 per cent F) causes a slight inhibition in growth. This is most noticeable in the males and particularly in the fourth and fifth generations. Otherwise there seem to be no cumulative effects of fluorine from generation to generation.

It is interesting to note that even the smallest amounts of fluorine caused typical changes in the teeth similar to those which have been adequately described in the literature (8, 4, 5, 10, 13, 7, 9).

Discussion. In general these results corroborate the work of earlier investigators in that they suggest an inhibition of growth in rats when fluorine is incorporated into the ration either as sodium fluoride or as rock phosphate. The levels at which we obtained our unfavorable results are similar to those of Sollmann and co-workers, and to those of Kick. They are somewhat below those reported earlier by Schulz and Lamb, and considerably lower than the levels used by McClure and Mitchell. The appearance and response of the animals under our experimental conditions to a level of 0.04 per cent calcium fluoride does not permit placing the toxicity of this substance on the same plane with that of sodium fluoride. It is more nearly in the ratio of that suggested by Sollmann and co-workers in comparing the toxicity of sodium fluoride to that of rock phosphate, or approximately that calcium fluoride is one-half as toxic as sodium fluoride.

The feed intake of our animals has not been kept except in the case of the higher levels (0.1, 0.2 and 0.3 per cent sodium fluoride). The intake, however, approximates about 1 gram of food per 10 grams of body weight for rats between 100 and 200 grams (or other limits). Thus the point where interference with growth occurs is near an intake of 18 to 20 mgm. of fluorine per kilo of body weight per day in the form of sodium fluoride and 36 to 40 mgm. of fluorine per kilo of body weight per day in the form of rock phosphate. Our experiments show further that 0.6 per cent rock phosphate is a safe level for rats insofar as growth is concerned. It seems therefore that 21 mgm. of fluorine per kilo of body weight per day is compatible with normal metabolism when the source of the fluorine is rock phosphate. A physiologically equivalent level of sodium fluoride would

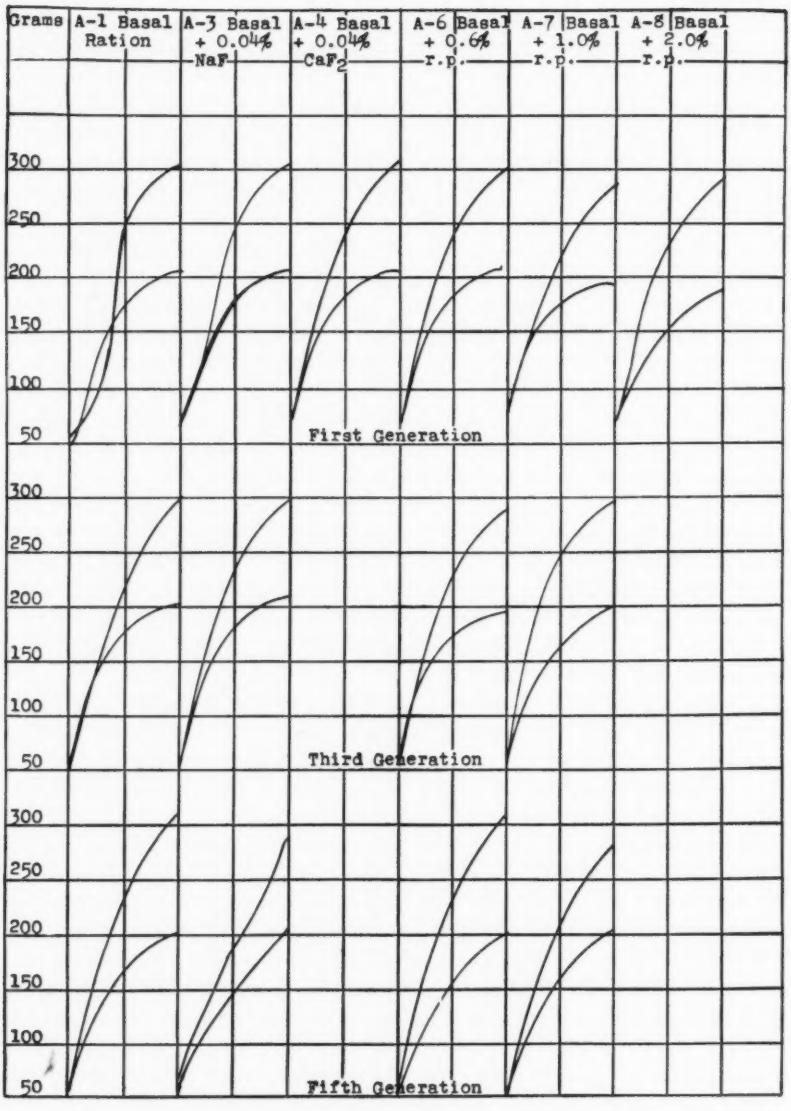

Chart 1. Growth curves, which are separate averages for the males and females in each lot.

be approximately 10 mgm. per kilo of body weight per day. These results essentially substantiate the early work of Sollmann *et al.*

The growth records indicate that the effects of fluorine action are felt most acutely during the suckling period. Apparently the levels used in these experiments are on the borderline of safety which still permits lactation but at the expense of the normal quantity or quality of milk. The slightly lower birth weights are further evidence that the metabolism of the fluorine-fed animals is somewhat impaired. It is interesting to point out that the 0.021 per cent fluorine level (0.6 per cent rock phosphate) is not sufficiently toxic to impair the growth of the young. Following the suckling period growth proceeds until average mature weights are attained, although somewhat more slowly.

CONCLUSIONS

The evidence obtained in these studies from birth weights, weaning weights, and growth records indicates that small doses of fluorine inhibit the normal growth of the rat. Daily intakes of approximately 20 mgm. fluorine per kgm. of body weight per day in the form of sodium fluoride and 40 mgm. fluorine per kgm. of body weight per day in the form of rock phosphate appear to be the upper limits of safety for growth in the rat. The toxicity of the fluorine in calcium fluoride is much less than that of the sodium salt and it seems to be of a similar magnitude to the toxicity of the fluorine in rock phosphate.

Typical effects of fluorine upon the incisor teeth are produced even at the lowest levels fed in these experiments, and this effect appears to be the most obvious and constant macroscopic index of fluorine toxicosis in the rat.

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STUDIES ON FLUORINE IN THE NUTRITION OF THE RAT[1]

II. Its Influence upon Reproduction

PAUL H. PHILLIPS, ALVIN R. LAMB, E. B. HART AND G. BOHSTEDT

From the Department of Agricultural Chemistry, and the Department of Animal Husbandry, University of Wisconsin, Madison

Received for publication July 14, 1933

In a previous paper (3) the importance of the study of chronic fluorine poisoning was pointed out and data were presented to show the influence of fluorine upon growth. The experimental evidence pertaining to its influence upon reproduction suggests that more information is needed. In this paper are presented the data obtained in a study of the mechanism of the effects of fluorine upon reproduction in the rat.

It has been observed by Schulz and Lamb (5) that fluorine has an unfavorable effect upon reproduction at a level of 0.025 per cent sodium fluoride, or more. Del Castillo (1) reported a suppression of the oestrous cycle when 0.05 per cent sodium fluoride was included in the ration. This observation harmonizes with that of Schulz and Lamb that reproduction is interfered with. McCollum and co-workers (4) secured reproduction in their experiments, which indicated that the females were fertile. Kick (2) concluded that high levels of fluorine in the ration have no direct effect upon reproduction in rats, but that there was evidence of a decrease in lactation. The evidence thus far indicates that a disturbance in reproduction does occur when animals receive small toxic doses of fluorine. The cause or mechanism through which this inhibition is brought about is not clear.

Experimental. Three series of experiments have been made in this study. The first, or "A" series, was an attempt to determine if fluorine had a cumulative effect upon reproduction and growth from generation to generation. The experimental plan for this series has been previously given (3).

The second, or "D" series, was designed to study the effect of fluorine upon oestrum and upon certain endocrine glands. In these experiments three lots of 20 females and 20 males each weighing about 50 grams were

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These studies were supported in part through a fellowship granted by the Ruhm Phosphate and Chemical Company of Mt. Pleasant, Tennessee.

placed upon the basal ration used in the "A" series (3). Lot D-1 received the basal ration only; D-3 the basal ration plus 0.043 per cent sodium fluoride, and D-7 received the basal ration plus 1.0 per cent rock phosphate. The males and females were kept in separate cages until breeding age which was arbitrarily set at 100 days. Vaginal smears were made daily from each female from the time of the opening of the vaginal orifice until removal from the experiment. The days during gestation and lactation were deducted from each individual smear record in averaging the data obtained upon the oestrous cycle. Each female was kept on the experiment sufficiently long for her to produce 1 or more litters and rear them to two weeks of age. When the animals were sacrificed the anterior lobe of the hypophysis cerebri and the suprarenal glands were quickly weighed. Histological studies, which will be presented later, were made on the ovaries, testes, thyroids, suprarenals, kidneys, and a few of the anterior lobes of the hypophysis. The remaining anterior lobes were transplanted subcutaneously into an immature female rat 22 to 24 days old. Two glands were transplanted the first day and two the following day into each recipient rat. The recipient rat was then killed on the fourth day and the ovaries weighed. The male rats were castrated 3 to 4 weeks prior to removal from the experiment in order to increase the gonad-stimulating potency of the anterior lobe of the hypophysis.

The third, or "H" series of experiments, was used to show the effects of sodium fluoride and of inanition upon the oestrous cycle. The basal "A" ration was used in these experiments. Vigorous mature females which had successfully reared one or two litters were used.

Ten such females were divided into two lots in the first experiment. Lot I with four females was given the basal ration throughout the experiment. Lot II with 6 females received the basal ration for 18 days and then the basal ration plus 0.15 per cent sodium fluoride for 33 days. During both periods daily vaginal smears were taken on both lots. The second experiment consisted of four lots and each contained four females chosen as in the previous experiment. They were fed as follows: lot I, basal ration only; lot II, basal ration + 0.15 per cent sodium fluoride; lots III and IV, the same rations as lots I and II except that 0.5 per cent yeast was added for the first 25 days and this was then increased to 4.0 per cent yeast for the rest of the period. Vaginal smears were taken for 28 days while all the animals were on the basal diet. Sodium fluoride was then added to the diet of the experimental lots and daily smears taken for 36 days. In both experiments the daily feed intake was carefully recorded for the fluorine-fed lots and the control lots were given an equal quantity of the basal ration and no more. The same observations and studies upon the endocrine glands and other organs were made as outlined for the "D" series.

RESULTS. *"A" series.* In the third generation A-3 females produced

only one litter each and then failed to produce subsequent litters. This also happened in lot A-7 in the fifth generation. Whether this can be attributed directly to fluorine action or to some other cause cannot be stated. This interruption in reproduction may have resulted from a chronic lung infection in these two lots, since several animals had to be discarded because of this disease.

It has been previously pointed out that the weights of the young at weaning time are lowered when suckled by mothers fed fluorine above 20 mgm. per kilo of body weight per day as sodium fluoride. This is apparently caused by a reduction in quantity or quality of the milk. That the daily fluorine intake for lactating mothers goes above the upper limit of safety for the rat is shown in table 1.

TABLE 1

Calculated fluorine ingested from daily feed records during lactation

	A-1 FEMALES BASAL RATION	A-6 FEMALES. BASAL RATION PLUS 0.6 PER CENT ROCK PHOSPHATE		A-3 FEMALES. BASAL RATION PLUS 0.43 PER CENT NaF	
	Daily feed intake	Daily feed intake	Daily fluorine intake (mgm. of fluorine per kilo of body weight)	Daily feed intake	Daily fluorine intake (mgm. of fluorine per kilo of body weight)
	grams	*grams*		*grams*	
First week of lactation.....	13.5	14.3	15.9	17.1	18.4
Second week of lactation....	19.0	18.9	21.0	20.0	20.1
Third week of lactation.....	21.1	21.6	23.9	20.0	21.6
Fourth week of lactation....	25.4	24.4	27.2	25.1	30.8

These data were taken from records kept on the 5th generation females and only females suckling 6 young were included. Thus the data were limited in numbers and the A-3 lot was represented by only one female. Usually the lactating females in lot A-3 (0.043 per cent sodium fluoride) and lot A-7 (1.0 per cent rock phosphate) became greatly emaciated and lost as much as 20 to 25 per cent of their body weight, while control females maintained their body weight, or nearly so, throughout the lactation period. It would seem that some metabolic disturbance occurred which seriously interfered with normal metabolism and that the effect was not specifically toxic to the reproductive or milk secretive functions.

Recovery of a thrifty, vigorous breeding condition was slow in the emaciated females, consequently more time was required to rebreed and reproduction was noticeably retarded in the A-3 and A-7 lots. Data presented in table 2 and further supported by data in table 3 indicate that retardation of reproduction was the only influence which can be attributed

TABLE 2

Influence of fluorine upon reproduction in successive generations

LOT AND RATION	NUMBER OF FEMALES STUDIED	TOTAL NUMBER OF LITTERS PRODUCED	AVERAGE NUMBER OF LITTERS PER FEMALE	AVERAGE SIZE OF LITTERS AT BIRTH
A-1 Basal A ration	3	11	3 7	7 4
A-3 Basal + 0.043% NaF	4	14	3 3	7 4
A-6 Basal + 0.60% r.p.	4	16	4 0	7 4
A-7 Basal + 1.0% r.p.	4	12	3 0	7 9
Second generation				
A-1 Basal ration	5	19	3 8	7 1
A-3 Basal + 0.043% NaF	4	10	2 5	5 5
A-6 Basal + 0.60% r.p.	3	12	4 0	7 3
A-7 Basal + 1.0% r.p.	5	18	3 6	5 9
Third generation				
A-1 Basal ration	3	15	5 0	6 5
A-3 Basal + 0.043% NaF	3	4	1 3	3 5
A-6 Basal + 0.60% r.p.	4	8	2 0	6 9
A-7 Basal + 1.0% r.p.	2	10	5 0	6 2
Fourth generation				
A-1 Basal ration	5	14	2 8	6 4
A-3 Basal + 0.043% NaF	3	5	1 7	7 6
A-6 Basal + 0.60% r.p.	2	5	2 5	5 6
A-7 Basal + 1.0% r.p.	4	7	1 8	6 6
Fifth generation				
A-1 Basal ration	5	18	3 6	5 9
A-3 Basal + 0.043% NaF	2	6	3 0	4 7
A-6 Basal + 0.60% r.p.	2	7	3 5	5 7
A-7 Basal + 1.0% r.p.	4	7	1 8	4 3

TABLE 3

Showing the influence of fluorine upon reproduction in the second series of experiments—D series

LOT AND RATION	NUMBER OF FEMALES STUDIED	TOTAL NUMBER OF LITTERS PRODUCED	AVERAGE NUMBER OF LITTERS PER FEMALE	AVERAGE SIZE OF LITTERS AT BIRTH
D-1 Basal A ration	16	18	1 1	6 0
D-2 Basal A + 0.043% NaF	20	19	1 0	6 0
D-3 Basal A + 1.0% r.p.	20	18	0 9	6 5

to fluorine action in these experiments. Thus the average number of litters per female was consistently lower in each generation than in the corresponding A-1 control females. The average size of litters at birth and the per cent of rats weaned were not appreciably influenced.

"D" series. Records of vaginal smears kept on twenty D-3 females for an average of 63.6 record days did not differ appreciably in the number of periods of oestrum from 16 control or D-1 females. The females in D-7 were likewise normal. Apparently at these levels of fluorine intake oestrum is not disturbed.

The gonad-stimulating potency of the implanted anterior lobes of the hypophysis in the fluorine-fed and the non-fluorine-fed animals showed no differences in the weight of the ovaries of the young recipient rats at the end of 96 hours. The ovaries in all lots were increased from 30 to 60 per cent in weight in the case of the animals used to test the potency of the

TABLE 4

Average fresh weights of the glands weighed in the D series

LOT AND DIET	NUMBER OF ANIMALS	WEIGHT OF ANTERIOR LOBE OF THE HYPOPHYSIS	WEIGHT OF THE SUPRARENAL GLANDS	MGM. OF SUPRARENAL PER 100 GRAMS BODY WEIGHT
		mgm.	*mgm.*	
Females, D-1 Basal ration	14	7.5	45.5	23.7
Females, D-3 Basal + 0.043% NaF	18	7.5	48.2	35.1
Females, D-7 Basal + 1.0% r.p.	17	7.0	46.8	25.7
Males, D-1 Basal ration	14	7.7	36.6	13.2
Males, D-3 Basal + 0.043% NaF	11	7.7	37.4	15.4
Males, D-7 Basal + 1.0% r.p.	14	7.5	41.0	16.6

female hypophyses and approximately 300 per cent in the case of the animals used to test the castrated males. Similar evidence was obtained on the gonad-stimulating potency of the anterior lobes from the "H" series females. Thus the evidence indicates that the gonad-stimulating potency of the anterior lobe of the hypophysis is not reduced by either moderate or fairly high doses of fluorine.

The average fresh weights of the endocrine glands are given in table 4. This table shows that there was very little variation in the fresh weights of the anterior lobes between lots, or sexes. The suprarenal glands seem to be consistently heavier in weight in the fluorine-fed animals than in the controls. It was also noted that the suprarenal glands were heavier in the females than in the males irrespective of the diet employed.

"H" series. The effects of inanition and sodium fluoride upon the oestrous cycle in the rat were strikingly similar as exhibited by the data in table 5. It was apparent that in both preliminary periods all the females

had a normal oestrous cycle occurring every 4.5 to 6.5 days. This lies well within the average range for the oestrous cycle in the rat. The body weights during the interval were nearly constant. It is pointed out that the control animals were held to a restricted food intake equivalent to that of the sodium fluoride-fed animals. When the experimental period began a sharp decline in frequency of oestrum was noted. This decline became particularly noticeable in the second week of the second experiment. The experimental and control animals behaved in identically the same manner and exhibited only an occasional oestrum. A loss in body weight was recorded

TABLE 5

Summary data showing the effect of restricted diets and sodium fluoride upon the oestrous cycle

LOT	PERIOD OF 18 DAYS ON BASAL DIET			PERIOD OF 33 DAYS ON EXPERIMENTAL DIETS			
	Initial weight	Number of oestral cycles	Frequency of oestrum	Initial weight	Average gain or loss in body weight for the period	Number of oestral cycles	Frequency of oestrum
First experiment "H" series (average per rat)							
	grams		*days*		*grams*		*days*
I. Basal ration		4.0	5.2		−13.8	3	16.5
II. Basal plus 0.15% NaF		3.5	5.3		−26.5	4	10.1
Second experiment "H" series (average per rat)							
I. Basal ration	224.5	5.5	5.7	213.5	+1.7	1.5	27.0
II. Basal plus 0.15% NaF	227.5	5.3	5.5	220.5	−24.5	1.8	16.0
III. Basal plus yeast	231.2	6.0	4.7	231.2	−17.8	2.0	15.3
IV. Basal plus yeast plus 0.15% NaF	239.5	5.0	6.3	224.5	−14.5	3.5	15.6

for each lot and furnished additional evidence that the feed intake was equal or nearly so in both groups.

This series furnished additional data upon the weights of the fresh suprarenal glands. The suprarenal glands increased in weight in the fluorine-fed lots over those of the controls. The average weight of the suprarenal glands for the control animals was 47.7 mgm. as compared to 53.1 mgm. for the fluorine-fed animals. There was no appreciable difference in the weights of the fresh anterior lobe of the hypophysis. These results were obtained upon fluorine intakes of from 26.8 to 29.4 mgm. of fluorine per kilo of body weight per day. This level of fluorine intake seems to be

sufficient to cause considerable reduction in the amount of food ingested. Since the control lots behaved in the same way as those receiving fluorine it seems that inanition caused the inhibition noted in the oestrous cycle. This may also account for the suppression of the oestrous cycle obtained by del Castillo (1).

Discussion. From the data obtained in these experiments it becomes increasingly obvious that fluorine toxicity has no direct and specifically unfavorable effect upon the reproductive mechanism in rats. The levels of fluorine intake near the upper margin of safety for the rat are without noticeable influence upon the oestrous cycle. When the intake goes distinctly above the upper limit of safety (20 mgm. of fluorine per kilo of body weight per day) to 25 or 30 mgm. of fluorine per kilo of body weight per day, a positive interference with oestrum is obtained. There is almost a complete cessation of oestrum. The evidence obtained in the "H" series of experiments shows quite clearly that inanition produces a similar if not identical result. It appears then that this interference with the oestrous cycle is probably caused by inanition.

The gonad-stimulating potency of the anterior lobe of the hypophysis is unimpaired by the amounts of fluorine fed in these experiments. In all cases a response in ovarian weight to the transplanted anterior lobe is noted. There is, however, no noticeable difference between the lots. This is taken as evidence that the effect of fluorine is not specific for the gonad-stimulating factor present in the anterior lobe of the hypophysis.

The fresh weights of the glands taken in these studies, although a gross index to be sure, are nevertheless of considerable interest. The average weights of the anterior lobe of the hypophysis are remarkably constant and fall within the narrow range of 6.95 to 7.70 mgm. per gland regardless of the dietary regimen. On the other hand a consistent difference is noted between the suprarenal weights of the experimental and the control animals. This is noticeable when 0.043 per cent sodium fluoride, or 1 per cent rock phosphate is included in the ration. The difference is not large but with few exceptions the fluorine-fed animals have heavier suprarenal glands. The differences noted in the "H"-series are more marked than those on the lower levels and suggest that the adrenal enlargement may be in a measure dependent upon the degree of toxicity encountered.

The results of these experiments yield considerable information on the effects of fluorine upon reproduction and the means by which the effect is produced. In view of the evidence obtained it seems justifiable to assume that there is no direct specific toxic action upon the reproductive organs and their functions. A moderate level of fluorine intake, or one near the margin of safety for the rat does not impair fertility, gestation, or parturition. The gonad-stimulating power of the pituitary gland remains the

same. Similarly the oestrous cycle is unaffected until the dosage exceeds the margin of safety and then only when it reaches a point where inanition occurs. The males are likewise unaffected by moderate levels of fluorine intake and maintain their fertility until highly toxic doses are given. No functional failure of lactation has been observed although the data are interpreted to mean that the toxicity of fluorine causes a reduction in milk secretion. Since the ingestion of fluorine usually increases to a definitely toxic level during lactation it is likely that the reduction in milk secretion may be due primarily to anorexia and subsequent cachexia. It seems that any toxic effect upon reproduction must operate, therefore, in an indirect manner.

Since no direct effect of fluorine has been demonstrated the question arises as to its specific mode of action. The generalized systemic reaction to chronic fluorine poisoning suggests that the mechanism of fluorine toxicity may be that of an interference in some manner with cellular metabolism.

CONCLUSIONS

Evidence is submitted to show that chronic fluorine poisoning does not inhibit reproduction in the rat, and therefore any unfavorable effect upon reproduction arises secondarily as the result of a systemic reaction to fluorine.

The oestrous cycle is not disturbed by low levels of NaF compatible with normal growth. If the dosage is raised beyond the threshold value of 25 mgm. of fluorine per kilo of body weight per day suppression of oestrum occurs. Inanition has been shown to be the cause of the suppression of oestrum when this amount of fluorine is fed as sodium fluoride.

The fresh weight of the suprarenal glands is increased by including fluorine in the ration. There is some evidence to show that the increase in weight of the glands is more pronounced when higher levels of fluorine are used.

Lactation is suppressed by fluorine when fed in amounts above the upper limit of safety for the rat. Similarly the rate of reproduction is sharply reduced when this limit of intake is exceeded. Coincident with this systemic action of fluorine is a reduced intake of food.

No clear-cut and positive evidence is available to show that chronic fluorine poisoning has a cumulative effect upon reproduction or other physiological processes from generation to generation for as long as five generations.

The gonad-stimulating potency of the anterior lobe of the hypophysis is unimpaired by the intakes of fluorine fed in these experiments.

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TEMPORAL AND SPATIAL SUMMATION IN AUTONOMIC SYSTEMS

A. ROSENBLUETH AND D. McK. RIOCH

From the Departments of Physiology and Anatomy in the Harvard Medical School

Received for publication July 17, 1933

It is well known that skeletal muscles can be considered as groups of motor units which may contract independently. Each muscle fiber receives a separate nerve supply and the excitatory process is localized, i.e., it is not transmitted from one muscle fiber to another.

Smooth muscle is organized differently. Histological evidence indicates that only some cells receive nerve endings (see Cannon, 1933, for references). The nerve impulse evokes the production of a chemical mediator (see Cannon, 1933) which diffuses into the blood stream and affects distant smooth muscle (Newton, Zwemer and Cannon, 1931). If the diffusion of the mediator were limited locally to certain groups of smooth-muscle cells, something akin to the motor units of striped muscle would ensue. If, on the other hand, there should be no local restrictions to this diffusion, smooth muscle would always respond as a whole, even when only a few of the nerve fibers distributed to it are stimulated.

Bishop and Heinbecker (1932) were unable to obtain a local contraction in the dilator of the iris when stimulating the cervical sympathetic submaximally. They came to the conclusion that the threshold response of the muscle as a whole is a function of the rate of nerve impulses delivered per fiber times the number of nerve fibers activated. This conclusion is incompatible with an organization into motor units and is in accord with a free diffusion of a chemical mediator.

The present paper deals quantitatively with temporal and spatial summation in smooth muscle as contrasted to skeletal muscle. The smooth muscle chosen was the nictitating membrane of the cat, an ideal preparation for quantitative observations: simple, accurate and relatively little subject to fatigue. The slowing of the heart rate on vagal stimulation was likewise examined from the same standpoint, because it was deemed desirable to extend the conclusions to other autonomic systems than smooth muscle.

METHOD. Cats were used, under dial anesthesia. Isotonic contractions of the nictitating membrane were recorded as described by Rosenblueth and Cannon (1931). The isometric contractions of the membrane

and of skeletal muscle were recorded optically by means of frictionless torsion-lever myographs (Cooper and Eccles, 1930). The heart beats were recorded from the thoracic wall by a Marey tambour.

The nerves were stimulated through shielded buried electrodes. In most experiments short (1σ) maximal shocks at varying frequencies were obtained from an improved "multivibrator" (see Rosenblueth, 1932a). In a few cases condenser charges (capacity 0.2 μF) were applied; the frequency was then regulated by a metronome. The stimuli were invariably recorded on a kymograph to insure accurate control of the frequency.

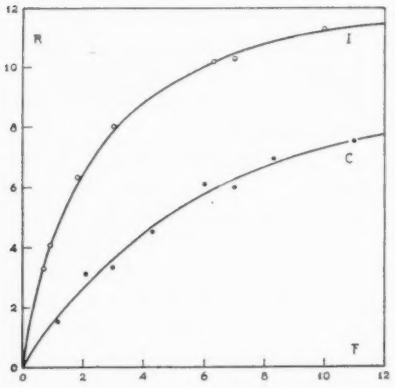

Fig. 1

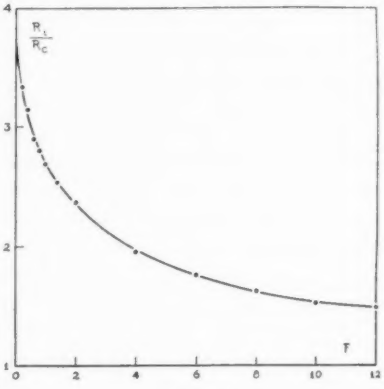

Fig. 2

Fig. 1. Isotonic contractions of the nictitating membrane on maximal peripheral stimulation of the cervical sympathetic in the neck at varying frequencies, I before, and C after cutting some of the branches of the superior cervical sympathetic ganglion.

Fig. 2. Ratios of the ordinates of curves I and C in figure 1, plotted against the corresponding frequencies.

The observations in a given series were performed in random order. The results were all consistent.

RESULTS. A. *Isotonic contractions of the nictitating membrane.* Series of responses to different frequencies of maximal stimulation of the cervical sympathetic in the neck were obtained before and after cutting some of the efferent branches of the superior cervical ganglion. The height of the responses in a given series was measured on the record at a constant interval after the beginning of the stimulus, usually from 10 to 15 seconds, sufficient for the contractions obtained from low frequencies (up to about 3 per second) to reach a steady plateau. This precaution is necessary, for the responses to higher frequencies do not present this plateau, but keep on rising (Rosenblueth, 1932b).

Typical results are plotted in figure 1. In this and the succeeding sections I and C denote respectively the curves obtained with the nerve supply *intact* and after some nerves were *cut*. The subscripts i and c are used to distinguish the symbols corresponding to the curves I and C.

As will be shown in the discussion, the following relations between the experimental curves I and C are important for the solution of the problem on hand: the ratios of the corresponding responses (R_i/R_c) at different frequencies (F) and the ratios of the frequencies (F_c/F_i) for different responses (R).

R_i/R_c is a function of the frequency, as illustrated graphically in figure 2. Similar results were consistently obtained. R_i/R_c is greater for lower than for higher frequencies and it tends to approach 1 as a limit—i.e., the responses tend to become identical at the higher frequencies, regardless of the number of nerve fibers cut.

F_c/F_i is, on the other hand, practically constant, as shown by the following typical values obtained from figure 1:

R	1	2	3	4	5	6	7
F_c/F_i	3.6	3.7	3.7	3.6	3.6	3.7	3.9

F_c/F_i is therefore not a function of the response. In words, the same ratio of higher frequency is necessary to obtain any given response after reduction of the nerve supply.

B. *Isometric contractions of the nictitating membrane.* In this case also, as for the isotonic contractions, varying frequencies of stimulation were plotted against the tensions developed in the corresponding responses. The precaution mentioned in the preceding section, of measuring the tensions after a constant period of stimulation, is likewise necessary.

Typical results are illustrated in figure 3. R_i/R_c varies here again with the frequency and tends to approach 1 as a limit with increasing frequencies, as shown in figure 4. F_c/F_i for different responses is again practically constant, as follows:

R	0.5	0.75	1.0	1.25	1.5	1.75	2.0	2.25	2.5
F_c/F_i	6.5	6.0	5.8	5.6	5.9	6.2	6.8	6.6	6.5

C. *Slowing of the heart rate on vagal stimulation.* The results are similar to those reported in sections A and B. Figure 5 illustrates two typical series of observations before and after cutting some of the fibers of the vagus in the neck, below the level of the electrodes. Maximal stimuli were applied at different frequencies for 20 seconds, and the number of beats occurring during the last 10 seconds of stimulation were counted. Slight variations in the basal rate were corrected by reducing the slowings

obtained to those corresponding to an average basal rate—this is equivalent to plotting per cent slowing. The variations in the basal rate are

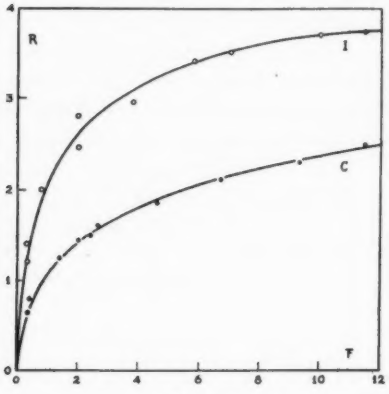

Fig. 3

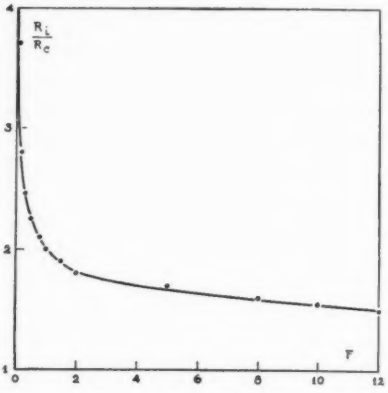

Fig. 4

Fig. 3. As in figure 1, but isometric.

Fig. 4. Ratios of the ordinates of curves I and C in figure 3, plotted against the corresponding frequencies.

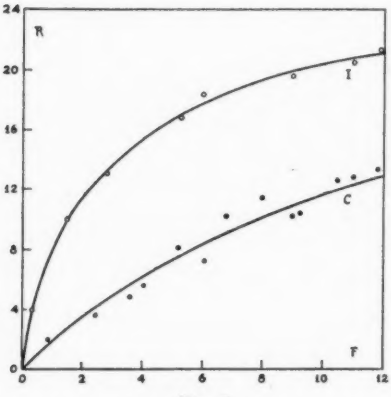

Fig. 5

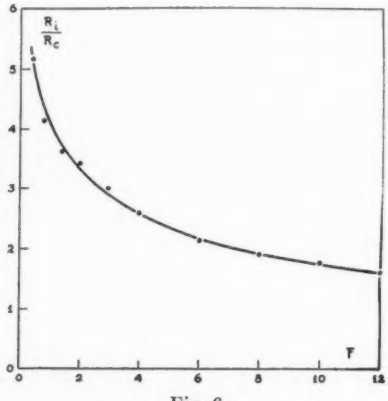

Fig. 6

Fig. 5. Heart-rate inhibition on maximal peripheral stimulation of the right vagus in the neck at varying frequencies, I before, and C after cutting some of the fibers of the nerve below the electrodes.

Fig. 6. Ratios of the ordinates of curves I and C in figure 5, plotted against the corresponding frequencies.

minimal under dial anesthesia, especially if the temperature is maintained constant.

The behavior of R_i/R_c at different frequencies is illustrated in figure 6. F_c/F_i for different responses is the following:

R.....	2	4	6	8	10	12	14	16
F_c/F_i.....	5.1	5.6	5.6	5.1	4.9	5.0	4.8	4.8

D. *Isometric contractions of skeletal muscle.* Control observations, similar to those reported in section B, were performed on the soleus and the gastrocnemius. The two muscles give analogous results. It is customary (see, e.g., Cooper and Eccles, 1930) to plot the maximum tension developed, i.e., the peak of the individual twitches in incomplete tetani. The level of summation is, however, probably more accurately represented by the

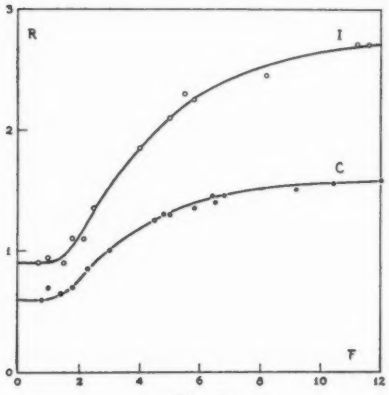

Fig. 7

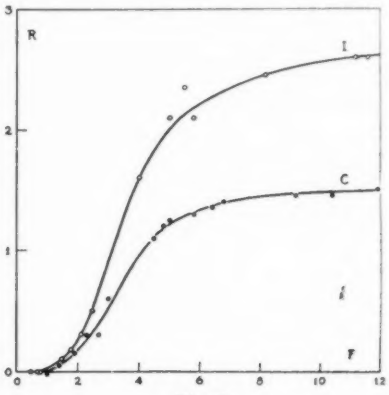

Fig. 8

Fig. 7. Isometric contractions of soleus on maximal stimulation of the motor nerve at varying frequencies, I before, and C after cutting some of the branches. Ordinates: highest tension developed at equilibrium in conventional units. Abscissae: frequency.

Fig. 8. As in figure 7, but ordinates: lowest tension developed at equilibrium.

persistent contraction on which the individual twitches are superimposed. Figures 7 and 8 illustrate the observations in a typical experiment on the soleus, plotted according to each of these criteria, respectively.

R_i/R_c is now constant, and not a function of frequency, as follows:

Curves in figure 7										
F.....	1	2	3	4	6	8	10	14	18	60
R_i/R_c.....	1.5	1.5	1.5	1.6	1.6	1.7	1.7	1.7	1.7	1.7
Curves in figure 8										
F.....	1	2	3	4	5	6	8	10	14	60
R_i/R_c.....	1.7	1.7	1.6	1.6	1.7	1.7	1.7	1.7	1.7	1.7

F_c/F_i, on the other hand, is here decidedly variable with the responses, increasing at first slowly and then rapidly as the response approaches the maximum of the C curve, as follows:

Curves in figure 7										
R	0.2	0.6	1.0	1.1	1.2	1.3	1.4	1.45	1.5	1.55
F_c/F_i	1.1	1.2	1.2	1.3	1.5	1.6	1.9	2.4	2.8	3.6

Curves in figure 8										
R	1.0	1.1	1.2	1.3	1.4	1.45	1.5	1.55	1.6	1.65
F_c/F_i	1.8	1.8	1.9	2.0	2.3	2.4	2.8	3.2	4.3	5.0

DISCUSSION. The consequence of cutting some of the nerve fibers—say, one-half—supplying a muscle organized as independent motor units will be to reduce the tension developed for any given frequency of maximal stimulation in the same proportion, i.e., one-half. The reasonable assumption is made that the motor units develop statistically similar degrees of tension for a given frequency of stimulation. The experiments performed on skeletal muscle confirm this statement (see figs. 7 and 8, section D). The ratio of the responses to any frequency before and after limiting the nerve supply is constant, and its value represents the ratio of the number of nerve fibers originally present to that of those remaining after the section.

Since smooth muscle does not show a constant fraction in the responses when a constant fraction of the original nerve supply is stimulated (sections A and B and figs. 2 and 4), it cannot be organized as motor units. Regardless of the number of nerve fibers cut, the ratio of the responses tends to approach 1 as the frequency increases (figs. 2 and 4). The maximum response available, therefore, is always the same, even after severe impairment of the nerve supply, within limitations imposed by the refractory period of the system. This can only be due to some mechanism whereby any nerve fiber supplying the muscle under consideration can influence all the muscle cells.

Smooth muscle has sometimes been described as a syncytium (see Evans, 1926, for references). It might appear reasonable to assume a physical excitatory wave traveling throughout the syncytium and thus bringing all the cells into play. A physical wave of excitation would be an all-or-none conducted disturbance. The assumption is, however, inadmissible, for the facts reported in sections A and B are strictly incompatible with any all-or-none interpretation. If an all-or-none excitatory wave stimulated all the elements of a syncytium the whole muscle would be a single motor unit with a plural innervation, and the responses to a given frequency of

stimulation would be identical before and after cutting some of the nerves, which is not the case. If there were, on the other hand, any subdivision into discrete groups presenting an all-or-none type of excitation, the situation would be identical with the motor units of skeletal muscle—i.e., the ratio of the responses would be constant. We are thus led to the conclusion that smooth muscle is not governed by the all-or-none principle, a conclusion already stated previously (Rosenblueth, 1932b).

The simplest explanation compatible with all the data available is that nerve impulses give rise to the production of a mediating substance which diffuses throughout the muscle, thus being capable of influencing relatively remote cells. The hyperbolic shape (see "Mathematical analysis," p. 373) of the curves obtained on different frequencies of stimulation (figs. 1 and 3) is explained by a chemical combination of this mediator (Rosenblueth, 1932b) with either excitatory or inhibitory receptor substances in the muscle cells (Cannon and Rosenblueth, 1933). The responses are lineally proportional to the concentrations of the compound formed.

According to the chemical hypothesis, the response is therefore a function of the concentration of the mediator. This concentration is built up by quanta, each nerve impulse from each fiber giving rise to a constant minute quantum of mediator—a consequence of the all-or-none character of the nerve impulses. Hence, the same amounts of mediator will be liberated with, say, twice the fibers discharging at half the frequency, as with half the fibers at twice the frequency. The experimental observations confirm this consequence, since the ratio of the frequencies is constant for any given response (sections A and B), while the ratio between the numbers of nerve fibers stimulated remains likewise constant. Bishop and Heinbecker's (1932) statement, quoted in the introduction, is thus found to be quantitatively accurate and may be extended as follows: the responses of autonomic systems are a function of the number of nerve impulses delivered per unit time, and not of the number of nerve fibers concerned. This characteristic of smooth muscle is hard to reconcile with any physical theory of excitation.

In the case of heart-rate inhibition it is difficult to conceive any discrete influence of each vagal nerve fiber on the effector, because of the nature of the response. One would therefore be led *a priori* to postulate a different mode of transmission of the nerve impulses than that occurring in skeletal muscle. It is interesting that the same interrelations between temporal and spatial summation exist in the heart as those found in smooth muscle (section C and figs. 5 and 6). The chemical theory accounts for this uniform behavior.

It will be shown in the mathematical analysis that, theoretically, if a sufficiently high frequency of stimulation is applied, a maximal response should obtain, even when the nerve supply has been considerably reduced.

In practice there are limitations to the theoretical prediction: the effective frequency of stimulation will be limited by the longest refractory period in the system; the concentration of the mediator will be too low for effective stimulation at a certain distance from the site of production, because of the diffusion gradient. In the experiment plotted in figure 1, however, a frequency of 18 per second gave a response of 8 cm. when only 27 per cent of the original nerve supply was effective after the section; this response is 77 per cent of the original, which was 10.4 cm. for the same frequency. If this behavior is contrasted with that of skeletal muscle under similar circumstances (fig. 7), a fundamental biological difference appears: a relatively considerable impairment of the nerve supply to smooth muscle or other autonomic systems produces only a small decrease in the responses, whereas damage of the nerve supply to skeletal muscle will involve a strictly proportional impairment of the responses.

Preganglionic neurones were sectioned in the vagus, but this does not invalidate the conclusions drawn, since, as shown by Bishop and Heinbecker (1932), autonomic ganglia are merely relays, and do not change the frequency of stimulation. Indeed, postganglionic fibers to the nictitating membrane were cut merely for convenience, for some observations performed by stimulating the thoracic sympathetic chains at different levels (and therefore different numbers of fibers) yielded identical results.

If isotonic responses of skeletal muscle were recorded, the resultant shortenings would not be proportional to the number of muscle fibers contracting. This is not the case, however, for smooth muscle where the elements are small enough so that the shortening is proportional to the contraction of the active cells, particularly since all the muscle participates in the response. That isotonic and isometric contractions of smooth muscle are quantitatively analogous is supported by the similarity of the results reported in sections A and B.

An immediate consequence of the independence of the motor units in skeletal muscle is that if there exists here, likewise, a chemical mediation of the nerve impulses the mediator does not diffuse outside the muscle cell in which it is produced. *A priori* we can then expect that any attempt to demonstrate directly this hypothetical mediator would fail unless some means were devised which would insure its diffusion. The fact that there is a summation of responses and that this summation reaches a definite submaximal equilibrium with an adequate frequency of stimulation strongly suggests the possibility of some excitatory mechanism similar to that occurring in smooth muscle, for this equilibrium is a serious objection to all the physical theories proposed to explain summation in muscle (see Rosenblueth, 1932b). One might feel tempted to reverse the process frequently employed in muscle physiology of applying to smooth muscle by mere analogy laws found to hold for skeletal muscle, and to state that, be-

cause nerve impulses are chemically mediated in smooth muscle, the same is probably true for the skeletal tissue. Certain it is that there is no known fact which excludes this possibility. The shape of the curves correlating tension at equilibrium with varying frequency of stimulation is compatible with the chemical theory, as will be shown below.

Mathematical analysis. As previously described (Rosenblueth, 1932b), the points of the series of observations plotted in figures 1, 3 and 5 fit adequately a hyperbola of the formula

$$R = \frac{F}{k + k' F} \qquad (1)$$

The constant k' is an index of the maximum response available, since it determines the horizontal asymptote of the hyperbola, which is $1/k'$.

The values of k and k', obtained by least squares, for the curves in figures 1, 3 and 5 are:

	k	k'	k	k'
Figure 1	0.185	0.088	0.613	0.097
Figure 3	0.238	0.255	0.8	0.34
Figure 5	0.1	0.039	0.5	0.036

The experimental data were tested with these values and the fit was found satisfactory. The value of k' as a rule was practically identical for any pair of I and C curves. This was likewise true when there were several C curves, i.e., when several series were taken involving different degrees of nerve section. The relatively large difference between k'_i and k'_c in figure 3 will be explained later when fatigue is discussed.

Since k' is constant for a given preparation, the maximal response available is always the same, notwithstanding impairment of the nerve supply. Hence, the whole muscle must participate in the response.

From (1)

$$\frac{R_i}{R_c} = \frac{k_c + k'_c F}{k_i + k'_i F} \qquad (2)$$

that is, another rectangular hyperbola. Figures 2, 4 and 6 should therefore fit this formula. If the fit is adequate
$(k_i/k'_i + F)\,(R_i/R_c - k'_c/k'_i) = (\mu + F)\,(R_i/R_c - \theta)$ should be constant.

Figure 2 gives the following test with the theoretical values for μ and θ, i.e., those derived from the constants of the curves I and C of figure 1: $(R_i/R_c - 1.1)\,(F + 2.1) = 5.06, 4.86, 4.93, 4.96, 5.21, 5.18, 5.34, 5.25, 5.2$.

The test for figure 4 is the following:
$(R_i/R_e - 1.33)\ (F + 1.28) = 2.2,\ 1.8,\ 1.6,\ 1.5,\ 1.6,\ 1.5,\ 2.3,\ 2.5,\ 2.5.$
The theoretical value of θ is the one employed; theoretically μ should be 0.933.

Figure 6 yields the following test:
$(R_i/R_e - 0.9)\ (F + 2.2) = 11.1,\ 9.66,\ 9.75,\ 10.6,\ 10.5,\ 10.4,\ 10.2,\ 10.4,\ 10.6,\ 10.3,\ 10.56.$
The theoretical values for θ and μ are 0.97 and 2.5 instead of 0.9 and 2.2 respectively.

Since it is assumed that free diffusion of the mediator occurs, we may write

$$R = \frac{q\,n\,F}{k + k'\,q\,n\,F} \qquad (3)$$

where q is the quantum of mediator liberated by each nerve impulse and n the number of nerve fibers stimulated. If the nerve is cut, n becomes n/r, i.e.,

$R = \dfrac{F}{\dfrac{k\,r}{q\,n} + k'\,F}$; and, since n and q are constant, we may incorporate them into k. Thus finally

$$R_i = \frac{F}{k + k'\,F} \qquad (4)$$

and

$$R_e = \frac{F}{k\,r + k'\,F} \qquad (5)$$

Consequently k' should not vary when the nerve is sectioned—it was shown above that this holds; and k_e should be $= k_i r$. The constant F_e/F_i represents, as we have stated, the value of n_i/n_e; it should be equal to $r = k_e/k_i$. This equality was invariably found to hold, except when fatigue occurred.

From (4) and (5), $\dfrac{R_i}{R_e} = \dfrac{k\,r + k'\,F}{k + k'\,F}$, which is identical with (2) if the constants are equal. The experimental data confirm the identity. As a consequence the curves in figures 2, 4 and 6 should cross the R_i/R_e axis at the point F_e/F_i, which was found to be the case in these and all other experiments.

It is important to note that the relations F_c/F_i = constant; and $\frac{R_i}{R_c} = \frac{\mu + F}{\theta + F}$ are not necessary consequences of the fact that the curves I and C are hyperbolas of the general formula (1). If $F_i = \frac{k_i R}{1 - k'_i R}$ and $F_c = \frac{k_c R}{1 - k'_c R}$ are any two such hyperbolas,

$$\frac{F_c}{F_i} = \frac{k_c}{k_i} \frac{1 - k'_i R}{1 - k'_c R}$$

$$\frac{d (F_c/F_i)}{d R} = \frac{k_c}{k_i} \left(\frac{k'_c (1 - k'_i R) - k'_i (1 - k'_c R)}{(1 - k'_c R)^2} \right).$$

For $\frac{F_c}{F_i}$ = constant, $\frac{d (F_c/F_i)}{d R} = 0$. But $k_c/k_i \neq 0$; and $(1 - k'_c R)^2 \neq \infty$. Hence, $k'_c (1 - k'_i R) = k'_i (1 - k'_c R)$; i.e., $k'_c = k'_i$. That is, F_c/F_i will only be a constant if $k'_c = k'_i$.

As regards R_i/R_c, formula (2) need not necessarily represent a hyperbola, for if $k'_i k_c = k'_c k_i$, R_i/R_c would be a constant $= k'_c/k'_i$.

It is thus shown that the experimental behavior of the ratios R_i/R_c and F_c/F_i is not a consequence of the hyperbolic shape of the curves of summation in autonomic systems, but a consequence of the fact that k' does not vary when some nerve fibers are cut, i.e., a consequence of the fact that any nerve fiber may influence the whole muscle, within the limitations previously described (p. 372).

Because of the lack of motor units, neither isotonic nor isometric responses of smooth muscle are lineally proportional to the number of nerve fibers stimulated at constant frequency. In our study on the distribution of thresholds in autonomic nerves (Rosenblueth and Rioch, 1933) we made this assumption when we substituted freely the number of nerve fibers for the responses.

This substitution is legitimate for isometric contractions of skeletal muscle, but cannot be performed for autonomic systems. The fractions of n activated by a series of stimuli of constant frequency and variable intensities or durations can, however, be readily calculated from the corresponding responses. From (3)

$$n = \frac{k R}{F (1 - k' R)} \qquad (6)$$

It is therefore sufficient to calculate k and k' from curve I. Figures 9 and 10 illustrate the changes which occur when n is thus calculated and substituted for R.

In figure 9 the vagus was stimulated with varying voltages at two different frequencies: 2 and 3.4 condenser charges per second. When plotted as per cent of the maximal, all the points in both the n and the R curves fall practically in the same line. Figure 10 is from an experiment on the nictitating membrane; the frequency was 25 per second. The efficiency of the formula used for the conversion is proved by the fact that n approaches an asymptote which is very close to the theoretical value of 1, which should obtain. In figure 9 this asymptote was 1.25, and in figure 10 it was 0.93.

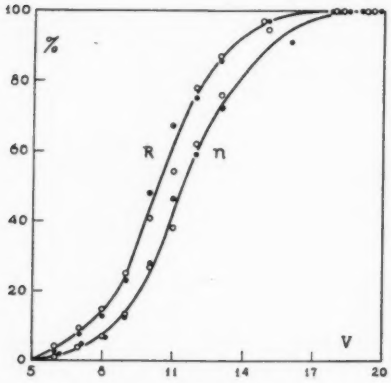

Fig. 9

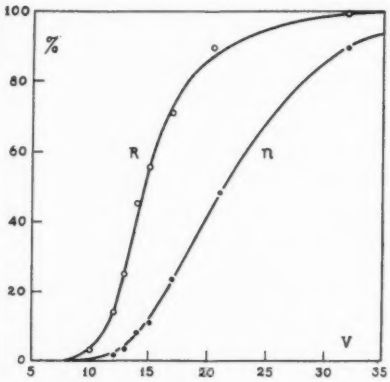

Fig. 10

Fig. 9. Conversion of per cent of heart-rate inhibition (R) into per cent of number of nerve fibers (n) activated. Circles: frequency of stimulation, 2 per second. Dots: frequency 3.4 per second. Ordinates: per cent R and n. Abscissae: volts. Condenser charges; capacity 0.2 μF; 10,000 ω in series with the nerve.

Fig. 10. Conversion of per cent of isotonic contraction (R) of the nictitating membrane into per cent of number of nerve fibers (n) activated. Abscissae: volts. Rectangular shocks of 1 σ; frequency: 25 per second; 200,000 ω in series with the nerve.

The lineal substitution of n for R in our paper (Rosenblueth and Rioch, 1933) does not affect any of the conclusions therein stated; it merely implies an erroneous graphic interpretation.

It was pointed out above that the experiment illustrated in figures 3 and 4 does not give as good a fit with the theory as was commonly obtained. In this experiment, shortly after the observations from which the curve I was drawn had been taken, consistently lower responses appeared for all frequencies. The nerve supply was intact; evidently fatigue had occurred. Although the preparation rested for about one hour, the muscle did not recover from the fatigue, and the curve C, taken later, is therefore complicated by this new variable. It was invariably found that if I is constructed from a fresh preparation and C after fatigue, k'_i is smaller than

k'_c. The k'_i before fatigue is likewise smaller than the k'_i after fatigue. The constant k', however, gradually reaches a practically constant value after fatigue has occurred, so that k'_i and k'_c are identical in a fatigued preparation.

If k' increases, the horizontal asymptote is lowered, i.e., the maximal response available is decreased. This is probably due to some change in the effector, not in the myoneural junction; for if q, for instance, decreased, k' would not be altered. We conclude, therefore, that fatigue of smooth muscle does not affect primarily the myoneural junction, but the contractile system.

At first sight, the shape of the curves of summation in skeletal muscle (figs. 7 and 8) differs greatly from those obtained from smooth muscle (figs. 1 and 3). They are really, however, closely related, as follows. In smooth muscle the chemical theory leads to the relation

$$R = \frac{(M)}{k + k'(M)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (7)$$

When we have substituted F for M and written $R = \frac{F}{k + k'F}$ we have assumed a lineal relation between (M) and F. This is a deliberate approximation which simplifies the formulation and is justified by the adequate fit which obtains (Rosenblueth, 1932b).

The mediator is not produced continuously but on the arrival of each nerve impulse, and there is a process which destroys M — hence relaxation. Assuming this destruction to be proportional to the concentration of M present at any given time,

$$(M) = q\,(e^{-C/F} + e^{-2\,C/F} + e^{-3\,C/F} + \ldots\ldots) = \frac{q}{e^{C/F} - 1} \quad \ldots\ldots (8)$$

at the time immediately preceding one of the nerve impulses after a sufficient number of these nerve impulses has been delivered at the frequency F; and

$$(M) = q\,(1 + e^{-C/F} + e^{-2\,C/F} + \ldots\ldots) = \frac{q}{1 - e^{-C/F}} \quad \ldots\ldots (9)$$

immediately after a nerve impulse has been delivered.

If we now substitute (8) and (9) for (M) in (7),

$$R = \frac{1}{k\,(e^{C/F} - 1) + k'} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (10)$$

and

$$R = \frac{1}{k\,(1 - e^{-C/F}) + k'} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (11)$$

The curves represented by (10) and (11) are similar to those in figures 8 and 7, respectively. If the theory is correct, the curves for summation in smooth muscle will then be identical with those obtained from skeletal muscle. They actually are, as the following considerations will show. Since the nictitating membrane gives a definite twitch to a single shock, its curve of summation, if plotted as in figure 7, should start above the origin and be convex towards the abscissae at the beginning. When plotted as in figure 8, however, it will begin at the origin. Complete tetanus occurs with such slow frequencies in smooth muscle that the two curves (10) and (11) are practically identical in almost all their path. This is also in keeping with the much greater ratio *maximal tetanus/maximal single twitch* in smooth muscle than in skeletal muscle.

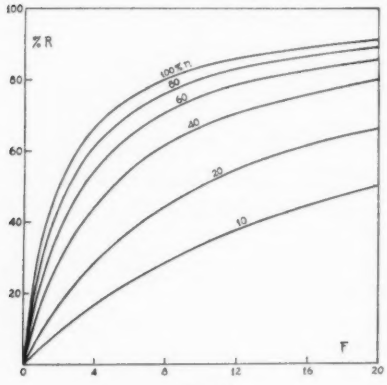

Fig. 11

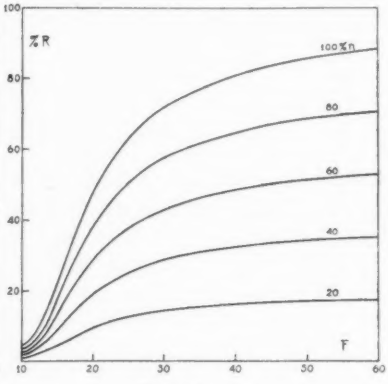

Fig. 12

Fig. 11. Theoretical relations between response (R), number of nerve fibers (n) and frequency of stimulation (F) in smooth muscle. For explanation see text.

Fig. 12. As in figure 11, but for skeletal muscle.

If C is small enough (M) becomes very soon practically proportional to F in (8) and (9). The curves (10) and (11) become then very approximately hyperbolas. If C is relatively large, on the other hand, this approximation to a hyperbola does not occur till F is relatively large. The constant C stands in these equations for the rate of destruction of the mediator. If the argument developed is correct, responses such as vagal cardio-inhibition, which disappear rapidly, should possess a small C and therefore deviate more from a hyperbola for the low frequencies than, e.g., the contractions of the nictitating membrane. This was found to be true in the present experiments and in those previously published (Rosenblueth, 1932b). Gilson (1933) reported also the same type of deviation.

The curves of summation in skeletal muscle are then such as would be

expected if there were a chemical mediation. The initial convexity toward the abscissae is more marked in faster white muscle than in the slower red tissue (cf. Cooper and Eccles, 1930). Lacking direct evidence on the matter, however, it is safer not to hazard a hypothesis.

Complete schematic pictures of the relations between F, R and n for smooth and skeletal muscle are presented in figures 11 and 12. Vertical slabbing of figure 12 (cf. Rosenblueth and Rioch, 1933), by making the frequency constant, will give a series of hyperbolas such as (6); while vertical slabbing of figure 11 yields straight lines: $n = cR$. Horizontal slabbing of figure 11 furnishes a family of equilateral hyperbolas referred to their asymptotes: nF = constant for any given R. Horizontal slabbing of figure 12, finally, gives complex curves which deviate more or less, according to the muscle chosen, from the equation $n(F - a) = b$ (a and b, constants). The product nF, which is equivalent to the number of nerve impulses per unit time, is constant for a given response in smooth muscle, whereas it is variable in skeletal muscle.

SUMMARY AND CONCLUSIONS

Isotonic and isometric contractions of the nictitating membrane (figs. 1 and 3), heart-rate inhibition (fig. 5) and isometric contractions of skeletal muscle (fig. 7), at varying frequencies of maximal stimulation, before and after severance of a fraction of the nerve supply, were recorded from cats.

The analysis of the curves thus obtained leads to the following conclusions:

a. Smooth muscle, unlike skeletal muscle, is not organized as motor units. In smooth muscle any nerve fiber of the motor supply can cause contraction of all the cells, within certain limitations (pp. 372–375).

b. Smooth muscle does not follow the all-or-none principle (p. 370).

c. The differences between skeletal and smooth muscle are explained by a free diffusion throughout the latter of a chemical mediator liberated by the nerve impulses (p. 371). This theory adequately covers other autonomic systems (heart-rate inhibition, p. 371).

d. The responses of autonomic systems are a function of the number of nerve impulses delivered per unit time, regardless of the number of nerve fibers involved, unlike skeletal muscle (p. 371).

e. A given destruction of the nerve supply impairs the responses of smooth muscle less than those of skeletal muscle (p. 372).

f. Isotonic and isometric responses of smooth muscle are quantitatively analogous, unlike skeletal muscle (p. 372).

g. The fraction of autonomic nerve fibers activated by a given stimulus can be determined from the response (p. 375).

h. Fatigue in smooth muscle probably affects first the contractile system, not the myoneural junction.

The possibility of a chemical mediation in skeletal muscle is discussed (pp. 372–377).

The relations between frequency of stimulation, number of nerve fibers activated, and response are analyzed mathematically both for smooth and skeletal muscle (p. 379 and figs. 11 and 12).

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STUDIES ON THE REGULATION OF GASTRIC ACIDITY

I. The Influence of Acid on the Secretion of Hydrochloric Acid by Fundic Pouches and by the Whole Stomach

CHARLES M. WILHELMJ, IRWIN NEIGUS and FREDERICK C. HILL

From the Departments of Physiology and Experimental Surgery, Creighton University, School of Medicine, Omaha, Nebraska

Received for publication July 15, 1933

At the present time there is considerable divergence of opinion regarding the mechanisms which regulate gastric acidity. In general all current theories can be grouped under two major headings, first, intrinsic or intragastric, and second, extragastric. The intragastric theories can again be subdivided into first, those which postulate an intragastric regulation of acidity by neutralization of acid, and second, those which explain the regulation of acidity on a physiochemical basis in which the concentration of hydrogen ions of the gastric contents determines the amount of acid secreted by the parietal cells. The present study is primarily an investigation of the latter theory, or more specifically, an attempt to answer the question of whether or not a certain concentration of acid within the stomach will inhibit secretion of acid by the fundic cells.

Pavlov was apparently the first to suggest that there is an automatic, intragastric mechanism controlling gastric acidity. He stated that when hydrochloric acid was allowed to accumulate in a fundic pouch it was found to inhibit further secretion of acid by the pouch. He believed that this inhibiting effect was specific for hydrochloric acid since phosphoric and butyric acid failed to inhibit the secretion of acid by a fundic pouch.

From a teleological viewpoint this theory is an attractive one and has gained several ardent supporters since it was first proposed by Pavlov. Evidence in support of this theory or some of its modifications has been presented by Roseman (1907), MacLean and Griffiths (1928), MacLean, Griffiths and Williams (1928), Apperly and Norris (1930), Apperly (1926), and Goldberg (1932). Another group of investigators comprising Bolton and Goodhart (1931–1933), and Hollander (1932) has prevented evidence against this theory.

In the present studies we have employed methods and calculations differing from those of the above investigators in the hope of gaining more information on this question.

METHODS AND PROCEDURE. Four dogs were employed in the experiments on the intact whole stomach. These animals had been used in studies on gastric secretion for nearly two years and were well trained and thoroughly accustomed to the various experimental procedures. There was no evidence of fear or even dislike for the various manipulations so that complicating psychic factors were probably eliminated. They were maintained upon a diet of dog biscuit and fresh whole milk with occasional meat feedings. All animals remained in perfect condition and either maintained the original weight or showed definite gains. They were routinely fed at 4:30 p.m. except on days preceding experiments when they were fed at 9:30 a.m.

The general plan of all experiments was to study the secretion of the intact, whole stomach or fundic pouch when filled with distilled water or with hydrochloric or other inorganic acid solutions. In all experiments the stomach or pouch was stimulated by an intramuscular injection of one milligram of histamin hydrochloride (synthetic).

In practically all of the previous investigations on this problem, the fractional method of gastric analysis has been employed, in which a given quantity of acid solution is placed in the stomach and allowed to remain there until the stomach empties; at frequent intervals small samples are withdrawn for analysis. While this method is capable of yielding valuable information it, nevertheless, possesses certain limitations and disadvantages.

We have employed the fractional method in a few experiments, but for the most part we have used what we call the "block" method of analysis. In general this method consists in introducing a definite amount of solution into the whole stomach or pouch (300 to 400 cc. in the whole stomach and 20 to 30 cc. in pouches). At the end of one half-hour the stomach or pouch is emptied as completely as possible and immediately refilled with fresh solution. At the end of the second half-hour period the stomach or pouch is again completely emptied and fresh solution again introduced. This procedure can be repeated as often as desired. Our routine procedure was to obtain three such half-hour samples covering a period of one and one-half hours after the injection of histamin.

The "Block" method was found to possess certain well defined advantages over the fractional method, first, it was possible to keep the stomach filled with a solution of more nearly constant composition; second, it provided a more accurate method of studying the emptying time of the stomach; third, since the volume removed each time represented the entire quantity of solution remaining in the stomach it was possible to more closely approximate the total quantity of chloride or acid secreted by the stomach during a given period of time in addition to knowing only the concentration as in the fractional method. When tenth normal hydrochloric acid solutions were employed the total quantity of fluid recovered

from the intact stomach averaged approximately 93 per cent of the quantity introduced, hence the calculations of the total secretion of the stomach were reasonably accurate; fourth, since with any given strength of acid solution the amount of fluid removed from the stomach was practically the same for each half-hour period, changes in volume during the one and one-half hour period after histamin injection do not alter the results as they so obviously do in the fractional method where the volume is decreasing during the entire experimental period; fifth, in the "Block" method it is much easier to establish a base line from which calculations are made than in the fractional method.

For introducing and removing the gastric sample we employed the apparatus designed in this laboratory by Moskowitz and Wilhelmj (1932). This apparatus has proven very satisfactory and no modifications have been necessary.

All experiments were performed approximately 24 hours after the last feeding. Before starting the experiment the stomach or pouch was lavaged one or more times with the test solution to be used. This lavage was always done in order to remove mucus and fasting secretion from the stomach, and thus to allow us to start from an approximate zero base line. The test solution was then introduced into the stomach or pouch and one milligram of histamin injected intramuscularly. Three one-half hour samples were obtained after the injection of histamin.

In most studies on gastric secretion and especially in the present problem it is absolutely essential to have some method whereby the amount of dilution of the solution introduced into the stomach or pouch can be accurately determined. This cannot be done, even in pouches, by merely recording the increase in volume of the solution, because this is never equal to the dilution since it is impossible to completely empty even small fundic pouches due to the fact that relatively large amounts of the solution are held in the rugae of the pouch wall. The discrepancy between the real dilution and the recorded increase in volume is usually quite large. The substance used to determine dilution must be one which is not destroyed by gastric contents and must not be absorbed from the stomach. Gorham (1923) was apparently the first to suggest the use of phenolsulphonephthalein (phenol red) for this purpose and it was subsequently used by Bulger, Stroud and Heideman (1928) in their studies on the electrolyte composition of gastric juice. We performed a series of control experiments in which acid solutions containing phenolsulphonephthalein were mixed with samples of gastric juice some of which contained bile and incubated at 37°C. for periods of from 6 to 48 hours. We found no change in the concentration of phenol red thus showing that this dye is not destroyed by gastric or mixed gastric and duodenal contents. In one group of experiments with tenth normal hydrochloric acid solutions in which almost none of the solu-

tion left the stomach during the experimental period we tested the urine for the dye and failed to find any after the solution had remained in the stomach for one and one-half hours. This and other indirect evidence indicates that the dye is not absorbed from the stomach under the conditions of these experiments. The concentration of phenol red in the test solution is a very important factor. If the concentration is too weak it is difficult to determine accurately and if too strong it may be impossible to detect relatively small dilutions. A series of control experiments showed that 12 milligrams of phenol red (subsequently completely dissolved in sodium hydroxide) in 1100 cubic centimeters of solution was a satisfactory concentration. In determining the concentration of phenol red in the samples removed from the stomach a portion of the original solution was used as the standard. After centrifuging, both the standard and unknown were made definitely alkaline by adding 1 cc. of saturated sodium hydroxide to 10 cc. of solution; both standard and unknown were then re-centrifuged and the colors compared in a colorimeter. Only on rare occasions did turbidity of the gastric sample interfere with the reading, when this occurred we removed the interfering substance (dissolved mucus) as follows: To 5 cc. of each standard and unknown solution add 2 cc. of an equal mixture of 10 per cent sodium tungstate and two-thirds normal sulfuric acid. Mix, shake and centrifuge. Decant the clear supernatant fluid. To 6.5 cc. of the standard and unknown solution, obtained by decanting, add 1 cc. of saturated sodium hydroxide and mix. A series of control experiments in which egg albumin solutions were added to the hydrochloric acid solutions containing phenol red showed that the removal of very much larger amounts of protein than were ever found in gastric samples, could be accomplished without loss of the dye. This method also removed bile pigments when present.

In most experiments the gastric sample was clear when alkalinized and centrifuged and it was not necessary to use the tungstic acid method. If bile pigments were present, however, it was not possible to accurately match the standard and unknown. It was found that a very small amount of picramic acid produced a change in color almost identical to that produced by bile. Under these conditions we would prepare two standard solutions and after alkalinization, add 1 or 2 very small crystals of picric acid to one. After mixing this solution would have a yellowish-red tinge. We would then mix small amounts of the picric acid standard with the unaltered standard until it gave a yellowish-red color equal to that produced by bile in the gastric sample. A series of control experiments showed that this procedure gave quite accurate results.

Neutral and total chlorides were determined after ashing by a modified Volhard procedure. The silver nitrate solution employed was equivalent to 1.5 mgm. of chloride per cubic centimeter. The precipitate of silver

chloride was removed by centrifuging before titrating the excess silver with ammonium thiocyanate. A few preliminary experiments convinced us of the necessity of ashing before determining chlorides in the samples. Direct determination, without ashing, gave grossly inaccurate results which were usually high, since even in these dilute samples there was sufficient dissolved protein (mucus) present to be precipitated by silver and render the chloride value inaccurate. The ashing was very carefully performed. It was found advantageous to add a small amount of sodium nitrate to the total chloride samples in order to hasten the ashing process. This could not be used in determining neutral chlorides. A series of control experiments was performed on standard hydrochloric acid solutions containing known amounts of sodium chloride with glucose as organic material. The experiments showed that our average experimental error was ± 3 mgm. of chloride and seldom exceeded ± 5 mgm. grams in individual experiments; that is, when the chloride equivalent of the titratable hydrochloric acid was added to the neutral chloride value as determined, the sum was equal to the determined total chloride value within the above limits. This balance was always calculated on every experimental sample in order to check the determination.

Calculations. When distilled water or inorganic acids other than hydrochloric were introduced into the whole stomach or pouches, it is obvious that all of the chlorides appearing in the sample were added by the various secretions which make up the gastric contents. When standard hydrochloric acid solutions were used certain calculations were necessary to determine how much of the various chloride fractions had been added by the secretions which make up the gastric contents.

These calculations are based upon the following general considerations: Any fluid which enters the stomach during the experimental period will cause a dilution of the test solution introduced into the stomach. The amount of dilution (number of cubic centimeters of fluid entering the stomach per 100 cc. of gastric contents) will be shown by the decrease in the per cent of phenol red in the sample removed from the stomach. The acid and chloride concentration of the acid solution originally placed in the stomach multiplied by the per cent of phenol red in the sample removed will correct the original solution for dilution; in other words, if the fluid entering the stomach were a neutral fluid containing no chloride, the acid and chloride concentration of the sample removed from the stomach would be equal to that of the original acid solution corrected for dilution. If the fluid which dilutes the phenol red contains chloride, the extra chloride added will be the difference between the chloride concentration of the original solution (corrected for dilution) and the chloride concentration of the fluid removed from the stomach. If the diluting fluid contains acid the extra acid can be calculated in a similar manner. If the fluid which

enters the stomach and dilutes the phenol red contains alkali, it will then be found that the acid concentration of the solution removed from the stomach is lower than that of the original fluid corrected for dilution, and the difference represents neutralized acid and the chloride equivalent of the neutralized acid will be present with the neutral chloride fraction. These few examples show the general principles on which the calculations are based. The details are as follows:

I. *Total extra chloride.* The total chloride concentration of the sample removed from the stomach minus (chloride concentration of the standard hydrochloric acid solution introduced x the per cent of phenol red in the sample) is equal to the total extra chloride. This represents the total chloride added by all fluids which enter the stomach during the experimental period and may include secreted hydrochloric acid, mucus, pyloric secretions and regurgitated duodenal contents.

II. *Neutral chloride.* This fraction is determined directly on the sample removed from the stomach, and is primarily sodium chloride. The total neutral chloride fraction includes sodium chloride brought in with the various secretions such as mucus, pyloric secretion, regurgitated duodenal fluids and also the neutral chloride resulting from the neutralization of hydrochloric acid.

III. *Acid chloride in samples removed from the stomach.* This is equal to the total chloride minus the neutral chloride.

IV. *Chloride of acid plus or minus corrected value.* The chloride concentration of the original standard hydrochloric acid solution introduced into the stomach $\times$ the per cent of phenol red in the fluid removed from the stomach gives the acid chloride concentration of the solution introduced corrected for dilution. If the acid chloride in the sample removed from the stomach is greater than this the difference represents the amount of acid secreted by the stomach which was not neutralized. If the acid chloride in the sample removed from the stomach is less than the amount in the original solution (corrected for dilution) the difference represents neutralized acid and the chloride equivalent will be present in the neutral chloride fraction.

V. *Chloride concentration of the various secretions comprising gastric contents.* One hundred minus the per cent of phenol red present in the sample removed from the stomach represents the cubic centimeters of fluid per 100 cc. of gastric contents which entered the stomach during the experimental period. This may include hydrochloric acid secreted by the stomach, mucus, pyloric secretions and regurgitated duodenal contents. The total extra chloride in the sample divided by the cubic centimeters of fluid entering the stomach gives the chloride concentration of these combined secretions.

Results. The present report is based upon approximately 148 experi-

ments performed during the past two years. One hundred six experiments were performed upon four normal intact dogs and 42 experiments were performed on 4 dogs with fundic pouches. One of the outstanding features has been the marked similarity of the results in all animals. In order to conserve space we will present only a few representative experiments and will mention any important differences which have occurred in other experiments.

A. *Fundic pouch experiments.* The fundic pouches were all of the Heidenhain type and held from 20 to 35 cc. Three were of the usual type in which one end of the pouch was brought out through a stab wound in the abdominal wall and one was made according to the method of Goldberg and Mann (1931) in which a piece of jejunum is connected to the pouch and brought to the abdominal wall. The latter type of pouch was not satisfactory for these studies since we found that there was considerable secretion from the intestinal loop which contained neutral chloride and we found it almost impossible to prevent the secretion from draining into the pouch and contaminating the contents with neutral chloride. All of the experiments reported here were performed on the remaining three pouches.

In no instance was a catheter allowed to remain in the pouch during the experiment nor was a cork used to close the opening of the pouch. Instead the test solution was introduced into the pouch through a soft rubber catheter which was immediately removed. The lips of the pouch were then firmly pressed together with the fingers and held during the experimental period. These precautions were taken in order to prevent irritation of the mucosa by a foreign body which might presumably result in an abnormal secretion of mucus in response to irritation. The pouches were stimulated by 1 mgm. of histamin injected intramuscularly.

The results of these experiments are shown in table 1 and figure 1.

Table 1 shows the chloride concentration of the secretion of fundic pouches under the following conditions: *1*, pure secretion collected from the pouch every half hour after histamin injection; *2*, when the pouches were filled with distilled water; *3*, when the pouches were filled with 0.101 normal HCl; *4*, when the pouches were filled with 0.173 normal HCl. There is no significant difference in the chloride concentration of the secretion obtained under these various conditions; nor are the differences which occur definitely related to the different solutions placed in the pouch. The differences which are seen are probably due to experimental errors and not to an actual change in the chloride concentration of the secretion. It will be shown later that the amount of neutral chloride present is insignificant in all of these experiments, hence the total chloride is practically all present as acid. The average value is 578 mgm. of chloride per 100 cc. of secretion which is approximately a 0.163 normal HCl solution.

Figure 1 shows the *actual* amounts of acid chloride, neutral chloride and

total chloride secreted by a fundic pouch when stimulated by histamin and filled with distilled water, 0.101 normal HCl, and 0.173 normal HCl. There are four characteristics of these experiments which deserve special emphasis: *1*, there is no evidence that the presence of acid in the pouch inhibits secretion of acid by the pouch, even when the acid in the pouch is of a higher concentration than the average value of the acid secreted;

TABLE I*

SOLUTION	POUCH I	POUCH II	POUCH III
Pure fundic secretion	560	578	
	556	561	
	551	507	
	526	566	
	568	566	
	567	553	
	548		
Average	554	555	
Distilled water	608	627	608
	596	578	590
	678	678	603
	579		
Average	615	628	600
0.101 N HCl	576	581	589
	585	556	605
	583	603	
Average	581	580	597
0.173 N HCl	522	577	
	544	581	
		599	
Average	533	572	

Average for 36 experiments = 578 mgm. per 100 cc.

* The chloride concentration of the secretion of fundic pouches filled with distilled water and hydrochloric acid solutions. Results expressed in milligrams of chloride per 100 cc. of secretion.

2, the neutral chlorides range between a maximum of 12 mgm. and a minimum of 0.6 mgm. and show no evidence of being increased when the pouch is filled with acid solutions; *3*, the neutral chlorides do not increase in amount as the acid secretion decreases as is practically always the case in the intact stomach; *4*, the neutral chlorides are always higher in the first half-hour sample and decrease to practically zero as secretion progresses. The pouches were always lavaged two to three times with the test solution

before the experiments were started but it is quite likely that not all of the thick tenacious mucus which covers the resting mucosa and contains neutral chloride was washed out, hence the neutral chlorides were higher in the first sample but were washed out as secretion progressed.

The curves in figure 1 represent the actual amount of the various chloride fractions secreted by the pouch and not the concentration per 100 cc. The latter method of expression often gives a very erroneous impression when the volumes recovered are small. For example, a neutral chloride concentration of 500 mgm. per 100 cc. in a volume of 3 cc. actually represents only 15 mgm. of neutral chloride. When such figures are being used

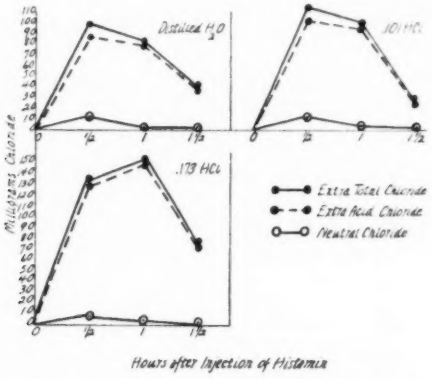

Fig. 1. Shows the actual amount of total extra chloride, extra acid chloride and neutral chloride secreted by a fundic pouch when stimulated with histamin and filled with distilled water, 0.101 normal HCl, and 0.173 normal HCl. The higher value obtained with the 0.173 normal HCl is of no significance since in some dogs the highest value was in the 0.101 normal HCl or distilled water. The variations are within the normal limit for secretion. Zero line represents the correction of the solution introduced for dilution.

to illustrate the magnitude of neutralization of hydrochloric acid the difference between the two methods of expression is of considerable importance but is frequently lost sight of. It is because of this distortion of concentration values in small volumes that we have plotted the actual values rather than the concentration per 100 cc. Table 2 gives other examples of this common fallacy taken from another experiment on a fundic pouch.

B. *Experiments on the intact stomach.* In these experiments the stomach was always lavaged thoroughly with the test solution immediately before the experiment was started in order to remove the fasting secretion and mucus and to allow the experiment to start from a zero base line. Three

hydrochloric acid solutions were used on each dog, namely, 0.024 normal, 0.058 normal, and 0.101 normal.

TABLE 2*

HCl solution containing 348 mgm. chloride per 100 cc. instilled into fundic pouch stimulated with histamin

TIME AFTER HISTAMIN	P.S.P.	CHLORIDE OF ORIGINAL SOLUTION CORRECTED FOR DILUTION	MILLIGRAMS PER 100 CC. IN SAMPLE REMOVED FROM POUCH					VOLUME OF SAMPLE	MILLIGRAMS ACTUALLY PRESENT IN SAMPLE			CHLORIDE CONCENTRATION OF FUNDIC SECRETIONS
			Total chloride	Neutral chloride	Total minus neutral	Total extra chloride	Extra acid chloride		Total extra chloride	Extra acid chloride	Neutral chloride	
	per cent							*cc.*				*mgm. per 100 cc.*
1st half-hour	46	160	471	59	412	311	252	18	56	45	11	576
2nd half-hour	39	136	493	38	455	357	319	25	89	80	9	585
3rd half-hour	65	226	430	21	409	204	183	22	45	40	5	583

* Experiment on a fundic pouch showing the marked difference between the actual amount of the various chloride fractions present and the concentration per 100 cc. when the volumes recovered are small.

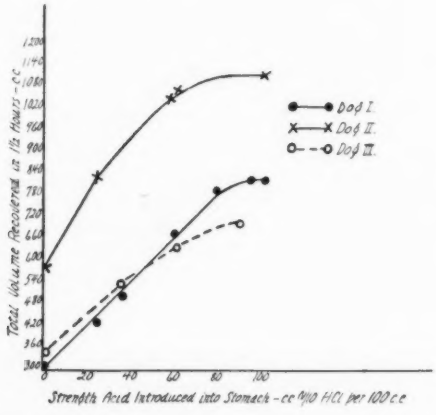

Fig. 2. Shows the total volume of solution recovered for a period of one and one-half hours after the injection of histamin when increasing strengths of hydrochloric acid solutions are placed in the stomach. In dogs I and III, 300 cc. and in dog II, 400 cc. of solution were introduced at the beginning of each half hour period and completely removed at the end of the period. Results represent the sum of the three one-half hour periods.

Practically all of these experiments were of the "Block" type in which 300 to 400 cc. of acid were introduced into the stomach at the beginning of each

half-hour period and the stomach emptied as completely as possible at the end of each half-hour period. It was found that the sum of the volumes recovered during the three half-hour periods, which constituted one complete experiment, increased progressively as stronger and stronger acids were introduced into the stomach. The increase in the volume recovered was strictly proportional to the strength of the acid introduced and represented 92 to 93 per cent of the amount introduced when tenth normal hydrochloric acid solutions were introduced. Figure 2 illustrates this in

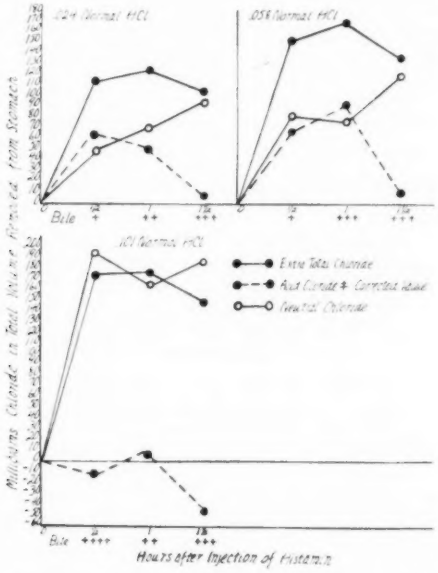

Fig. 3. Shows the amount of extra total chloride, acid chloride ± the corrected value and neutral chloride in the total volume removed from the stomach when the stomach was filled with 0.024, 0.058, and 0.101 normal HCl solutions. The increase in the amount with stronger acids is due to the greater volumes recovered. The zero line represents the correction of the original solution for dilution.

three dogs in which we began with distilled water and progressively but slowly increased the strength of acid up to tenth normal.

Figure 3 and table 3 show the results obtained when increasing strengths of hydrochloric acid solutions were introduced into the intact whole stomach stimulated by histamin. In figure 3 (dog I) the results are given in terms of the total quantity of the various chloride fractions in the gastric samples. In table 3 (dog II) they are given in the usual manner, i.e., in milligrams per 100 cc. In these experiments where the

volumes are large the latter manner of expressing the result is not as likely to give an erroneous impression as when the volumes are very small. A

TABLE 3*

HOURS AFTER HISTAMIN	STRENGTH ACID	P.S.P.	MILLIGRAMS PER 100 CC.						CHLORIDE OF SECRETION	VOLUME	BILE	REMARKS
			Total chloride	Neutral chloride	Total minus neutral	Chlorides corrected for dilution	Chloride of acid ± corrected value	Total extra chloride				
		per cent										
½	0.024 N	87	142	24	118	69	+49	73	561	334	0	
1	79 mgm. Cl per	81	183	33	150	64	+86	119	627	289	VF+	
1½	100 cc.	87	133	47	86	69	+17	64	492	174	+	
1½	0.024 N	82	156	24	132	65	+65	91	506	403	0	
1		73	193	20	173	58	+115	135	500	246	0	
1½		82	144	34	110	65	+45	79	439	222	VF+	
½	0.058 N	83	241	35	206	164	+42	77	453	352	+	
1	197 mgm. Cl	78	274	29	245	154	+91	120	545	282	VF+	
1½	per 100 cc.	83	265	27	238	164	+74	101	594	290	VF+	
½	0.058 N	87	242	20	222	172	+50	70	538	420	0	
1		78	277	47	230	154	+76	123	559	356	+	
1½		87	238	44	194	172	+22	66	507	304	+	
½	0.101 N	85	371	29	342	296	+46	75	500	354	+	
1	348 mgm. Cl	83	382	34	348	289	+59	93	547	446	+	
1½	per 100 cc.	86	374	35	339	299	+40	75	536	402	+	
½	0.101 N	83	366	26	340	288	+52	78	459	430	+	
1		81	380	22	358	282	+76	98	516	396	0	
1½		81	350	82	268	282	−14	68	358	406	+++	
½	0.101 N	86	365	41	324	299	+25	66	471	406	+++	
1		86	373	25	348	299	+49	74	528	404	0	
1½		81	347	124	223	282	−59	65	342	240	++++	Vomited
½	0.101 N	84	370	26	344	292	+52	78	488	432	0	
1		77	383	38	345	268	+77	115	500	410	VF+	
1½		83	362	103	259	289	−30	73	430	90	++++	Vomited

* A complete series of experiments on dog II in which increasing strengths of hydrochloric acid were placed in the stomach. Stomach stimulated with histamin.

word must be said here regarding the frequency of regurgitation of duodenal contents. The four dogs used in these experiments showed this

phenomenon in varying degree. Dog I (fig. 3) showed considerable regurgitation from the duodenum and it seemed to become more marked as stronger acids were placed in the stomach. Dog II (table 3) showed an intermediate degree of regurgitation while dogs III and IV showed relatively little.

Figure 3 and table 3 show quite clearly that the extra total chloride added to the acid solution while in the stomach is not decreased as stronger acids are placed in the stomach. The slight rise with increasing strength of acid shown in figure 3, where the results are calculated on a total volume basis, is due to the greater volume recovered with the stronger acids, the slight drop shown in table 3 where the results are given in terms of concentration, is due to the same phenomenon. When the results are calculated on a total volume basis, the experiments with the tenth normal hydrochloric acid solution represent practically the entire secretion of the stomach. With the 0.024 normal and 0.058 normal acids the true secretion is higher than shown because much more of the solution had left the stomach during the half-hour period. When approximate corrections are made for these differences in the volume recovered there is no essential difference in the amount of extra chloride added when the different strength acid solutions are placed in the stomach.

The amount of extra acid chloride which is present in the samples recovered from the stomach is obviously a balance between the amount of acid secreted by the stomach and the amount of acid neutralized. If neutralization is very great, little or no extra acid chloride may appear in the samples but the neutral chlorides will be correspondingly high, from this alone it would be erroneously concluded that no acid was secreted by the stomach but that a corresponding amount of neutral chloride was secreted. This is illustrated in the experiments with 0.101 normal HCl shown in figure 3. Because of the fact that there is an increasing amount of regurgitation from the duodenum as stronger acids were placed in the stomach, the acid chlorides show a progressive decrease. In the experiments shown in figure 3 if the extra acid chloride alone was considered it would be logical to conclude that 0.101 normal HCl caused almost complete inhibition of acid secretion. It should be emphasized that in figure 3 the zero line represents the correction for dilution so that when the acid chlorides are below this line it represents neutralization because there is no evidence that acid is ever absorbed from the stomach and also because as the acid chlorides decrease the neutral chlorides increase. In dog II (table 3) in which relatively little regurgitation occurred it is quite obvious that acid secretion was not inhibited when 0.101 normal HCl was placed in the stomach. In two of the third half-hour samples with the 0.101 normal HCl the dog vomited and coincident with this there was a drop in the acid chlorides below the level corrected for dilution and an abrupt and coincident elevation of

the neutral chlorides, these changes being accompanied by a marked increase in the bile content of the samples.

The experiments in figure 3 and table 3 show very clearly that there is an inverse relationship between the acid chlorides and the neutral chlorides so that as the acid chlorides fall the neutral chlorides rise. When these results in the intact whole stomach are compared with the results obtained in fundic pouches (fig. 1) it is clear that this inverse relationship between acid and neutral chlorides is not a function of the fundic part of the stomach. We must therefore conclude that this inverse relationship between acid and neutral chlorides is a function of either or both the pyloric part of the stomach and the duodenal fluids. In table 3 and figure 3 it is seen that the bile content of the samples approximately parallels the neutral chloride content, thus suggesting that duodenal fluids are in some way responsible for the change. In dog I (fig. 3) this was beautifully proven by ligation of the pancreatic and bile ducts after which large amounts of extra acid chlorides appeared in the samples when the 0.101 normal HCl solution was used, so that the dog then resembled dog II (table 3). The inverse relationship between the acid and neutral chlorides was also abolished by ligation of the pancreatic and bile ducts.

The chloride concentration of the combined secretions which enter the intact stomach is in general lower than the value obtained in fundic pouches. In general the greater the amount of bile in the samples the lower the concentration and conversely when bile is absent the chloride concentration approaches that obtained in fundic pouches. This is well illustrated in column 10, table 3. In general this was true in all experiments. The average value for the chloride concentration of the secretions entering the stomach in dog I (fig. 3) was 460 mgm. grams per 100 cc.; in dog II (table 3) it was 513 mgm. per 100 cc. and in dog III, 559 mgm. per 100 cc. Regurgitation from the duodenum in these three dogs varied in an inverse manner being greatest in dog I and least in dog III. These results suggest that the fluids which regurgitate from the duodenum have a lower chloride concentration than that of pure fundic secretion. A more detailed analysis of these facts will be presented at a later date.

A large series of experiments was performed on three of the normal dogs in which we employed solutions of sulfuric acid, nitric acid and sulfuric acid plus sodium chloride in concentrations up to 500 mgm. of chloride per 100 cc. Another series was performed with aqueous solutions of sodium chloride in various concentrations in order to investigate the influence of osmotic pressure and of chloride ions on gastric secretion. None of these experiments gave any evidence of inhibition of secretion of acid by the stomach and can be dismissed without further comment.

Discussion. The experiments with fundic pouches clearly illustrate four important facts: 1, the concentration of hydrochloric acid secreted by the fundic cells is not altered when the pouch is filled with hydrochloric

acid solutions, even when the solutions are stronger than the acid secreted by the pouch; 2, the presence of hydrochloric acid in the pouch does not cause a decrease in the total amount of acid secreted by the pouch; 3, neutral chlorides do not increase in amount when the pouch is filled with hydrochloric acid of increasing strength; 4, neutral chlorides do not increase in amount as the curve of acid secretion falls, as is the case in the intact stomach.

The first three findings are in direct opposition to the theory of the control of acid secretion proposed by MacLean and his co-workers who are the chief modern supporters of this theory. According to this theory when hydrochloric acid solutions are placed in the stomach or when the acid secretions of the stomach are allowed to accumulate, as the strength of acid reaches a certain level the fundic cells cease to secrete hydrochlorid acid but continue to secrete a neutral fluid rich in mineral chlorides which reduces the acidity of the gastric contents by dilution; when the acidity has been reduced below a certain level the fundic cells may again begin to secrete hydrochloric acid. This is really a modification of the older theory of Rosemann (1907) which postulates that the parietal cells are able to secrete either hydrochloric acid or sodium chloride in varying proportions according to the intensity of the stimulus. MacLean, Griffiths and Williams (1928) believe that they have demonstrated this theory in fundic pouches as well as in the intact stomach. Goldberg (1931) has also claimed that the presence of hydrochloric or sulfuric acids in fundic pouches inhibits the secretion of acid by the pouch but he concludes that dilution by a fluid rich in neutral chloride is a minor factor. The data shown in figure 1 do not support this theory. The data obtained on the intact normal stomach (fig. 3 and table 3) also show that there is no decrease in the amount of extra chloride added to acid solutions placed in the stomach. In dog I (fig. 3) the increase in neutral chlorides which occurred when strong acid solutions were placed in the stomach is not due to the fact that the parietal cells cease to secrete hydrochloric acid and continue to secrete sodium chloride but is due largely to regurgitation from the duodenum. Dog II (table 3) which showed only a small amount of regurgitation from the duodenum did not show an increase in neutral chloride unless there was a large amount of bile in the gastric samples. It appears that these data, which were verified in 106 experiments on 4 normal dogs and 36 experiments on 4 fundic dogs, are sufficient to definitely disprove the Rosemann-MacLean theory of the control of gastric acidity.

Without entering into a detailed discussion of the results of MacLean and his co-workers and of Goldberg we will simply state that their interpretations are the result of the failure to correctly evaluate (MacLean) and to correctly determine (Goldberg) the factor of dilution of the acid solution placed in the stomach. This can be illustrated from our data. In table 3, column 4, are shown the total chloride concentrations of the

samples removed from the stomach. If for each strength of acid introduced into the stomach, the chloride value of the respective acid, i.e., 79, 197, and 348 mgm., be substracted from the total chloride value of the samples removed from the stomach, the result will be a perfect picture of increasing inhibition as stronger acids are introduced. It is only when the amount of the original acid solution which still remains in the stomach is determined by the per cent of phenol red present that the true amount of acid secreted by the stomach can be accurately determined. Apperly (1926), while he believed in the acid inhibition theory was, nevertheless, more nearly correct in his interpretation of the dilution factor than the above mentioned workers.

The values which we obtained for the chloride concentration of the secretion of fundic pouches are in good agreement with the values reported by other investigators. Gamble and McIver (1928) found that the chloride concentration averaged 586 mgm. per 100 cc. and varied from 536 to 628 mgm. Hollander (1932) from a mathematical analysis of the data obtained from fundic pouches found that the maximum acidity of the secretion of the parietal cells averaged 592 mgm. of chloride per 100 cc. and ranged from 577 to 613 mgm. The excellent agreement of our values with those of these investigators fully justify the calculations and methods which we have employed.

We believe that it is important again to stress the fact that there is no evidence of a change in the concentration of hydrochloric acid secreted by fundic pouches even when filled with 0.173 normal HCl, a strength which never exists in the intact stomach. This fact definitely proves that a change in the concentration of hydrochloric acid secreted by the fundic cells is not one of the factors regulating gastric acidity.

In the intact stomach the chloride concentration of the secretion is definitely lower than that of fundic pouches. A study of our data shows that in general the greater the amount of bile present the lower the chloride concentration of the combined secretions constituting the gastric contents. This suggests that the chloride concentration of the regurgitating duodenal contents is lower than that of the fundic secretions.

The influence of increasing strengths of acid on the emptying time of the stomach (fig. 2) is in agreement with the results recently reported by Stewart and Boldyreff (1932). This observation was also made by Thomas (1931) and was incorporated in his theory of the mechanism of gastric evacuation. Our results show that this mechanism is very delicately adjusted to even small changes in hydrogen ion concentration. Sulfuric acid solutions of similar hydrogen ion concentrations were also found to fall on these curves for hydrochloric acid. In agreement with Stewart and Boldyreff we have also noted that sodium hydroxide solutions cause a pronounced increase in the emptying time.

SUMMARY

1. Hydrochloric acid solutions ranging in strength from 0.024 to 0.173 normal do not inhibit the secretion of acid in fundic pouches or in the whole stomach. The same is true for nitric and sulfuric acids alone or in combination with sodium chloride.

2. When these hydrochloric acid solutions are placed in fundic pouches there is no increase in the amount of neutral chloride secreted by the pouch. The amount of neutral chloride in fundic secretion is very small and does not show the inverse relationship to the acid chloride that is found in the intact stomach.

3. The chloride concentration of fundic secretion was found to average 578 mgm. per 100 cc. This is approximately a 0.163 normal HCl solution. The concentration of hydrochloric acid secreted by fundic pouches is not altered by placing hydrochloric acid solutions up to 0.173 normal in the pouch.

4. The chloride concentration of the combined secretions (fundic, pyloric and regurgitated duodenal contents) found in the intact stomach is definitely lower than that of pure fundic secretion and the lowering is proportional to the amount of bile present in the gastric sample.

5. When increasing strengths of hydrochloric and sulfuric acids are placed in the stomach there is a progressive increase in the emptying time which is strictly proportional to the strength of acid introduced into the stomach.

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THE PROGRESSIVE DEGENERATION OF FROG NERVE

G. H. PARKER

From the Zoölogical Laboratories, Harvard University

Received for publication July 17, 1933

In a forthcoming paper on the degeneration of the lateral-line nerve of the catfish (Parker and Paine, 1933) it has been shown that degeneration in this nerve when tested histologically is *progressive* in character and does not take place simultaneously throughout the whole length of the nerve. This progressive phase of the process is best seen in the lateral-line nerve from about the thirteenth to the fifteenth day after the degenerative cut has been made. During this period the changes in the neurofibrils and in the medullary sheaths are unquestionably more advanced in the anterior than in the posterior part of the nerve and thus give evidence of progressive alterations. If cold-blooded nerve when studied histologically can in this way show a progressive type of degeneration, it seems fair to expect that physiological evidence of such a change might also be obtainable. To test this, degeneration experiments were carried out on the sciatic nerves of the common spotted frogs of New England, Rana palustris Lec. and Rana pipiens Schr.

In this work frogs were anesthetized by being placed in ice-cold water for some fifteen minutes. A longitudinal incision a few millimeters in length was then made through the skin of the frog between the posterior portion of the urostyle and the ilium. The subjacent thin layer of muscle between these two bones was then cut, and, by means of a small tenaculum inserted in the aperture, the sciatic nerve was lifted to the surface of the wound and transected. The two cut ends of the nerve slipped back into place, and the wound closed over of itself. The frog was then put into an aquarium with shallow water whose temperature was maintained at about 18°C. Usually the cut was made on the right side of the frog, the left being reserved in normal state as a check. The great majority of frogs stood the operation well and survived for the periods necessary for the tests. In many animals the wounds after a week or two healed completely. The maximum length of time during which the frogs were kept after the operation was about 20 days. The operated frogs swam and hopped about freely with their three normal legs, the denervated leg being moved only at the level of the hip.

Frogs were treated in the way described in lots of about fifteen to twenty.

Beginning at a definite time after the operation, a few of the frogs were killed day by day and the condition of their cut sciatic nerves compared with that of their normal ones. Ordinarily the two nerves were exposed throughout the length of the thigh and their central ends stimulated by a weak tetanizing current. The conditions of the nerves were judged by the motor responses of the two feet. The stimulating current was taken from a Harvard inductorium activated by a single Columbia dry cell, with the secondary coil at 12 cm. from the primary and at an angle of about fifteen degrees from the vertical.

Early workers on the degeneration of frog nerves have studied the subject almost exclusively from a histological standpoint (Lent, 1856; Neumann, 1868, 1880; Hertz, 1869; Eichhorst, 1874; Engelmann, 1876; Colasanti, 1878; Mönckeberg and Bethe, 1899), but a few have added more obvious physiological records (Erb, 1869; Bethe, 1903; Weiss as

TABLE 1

Table of days, from the first to the twentieth after the degenerative cut, on which the following states of the sciatic nerve of the frog were observed: 1, *no degeneration*, reactions from cut nerve indistinguishable from those from normal nerve; 2, *part degeneration*, reactions from cut nerve less than from normal nerve; 3, period of *progressive degeneration;* 4, *full degeneration*, no reaction from cut nerve. 18°C.

	NUMBERS OF DAYS																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1. No degeneration	x	x	x	x	x	x														
2. Part degeneration					x	x	x	x	x	x	x	x	x	x	x	x				
3. Prog. degeneration												x	x	x	x					
4. Full degeneration																x	x	x	x	x

quoted by Courrier, 1926). Their contributions will be considered in the following account.

In the early stages of the tests, frogs were found from the first to the sixth day inclusive in which on stimulating first the cut and then the normal nerve no difference could be detected in the muscular responses of the foot. Thus physiological evidence of degeneration appeared to be absent up to the sixth day. Nerves that called forth some response but less than the normal nerve did, thereby indicating part degeneration, were found from the fifth to the sixteenth day inclusive. Complete absence of muscular response, indicating what might be called full physiological degeneration, was observed in most instances from the sixteenth to the twentieth day (table 1). As already noted my observations ordinarily extended only to the twentieth day. It can, however, be stated that in one particular instance complete physiological degeneration was observed at the twenty-seventh day and that at that time no sign of regeneration had as yet appeared.

These time relations are in fair accord with those already noted in the literature. Loss of function, probably incomplete, was observed by Erb (1869) in the leg nerves of a frog at the ninth day and at the twentieth day after the nerve had been crushed. Bethe (1903, p. 165) noted the failure of transmission over a frog nerve severed thirteen days previously. Weiss, according to Courrier (1926), found that loss of muscle response appeared in frog nerve after 13 days at 16°C. and after 35 days at 8°C. These few records, when the temperatures are taken into account, are in reasonable agreement with the more extended set reported in this paper.

The real problem that we have before us, however, is not the times at which nerve transmission changes but whether this change affects a given nerve simultaneously throughout its length or progressively. To ascer-

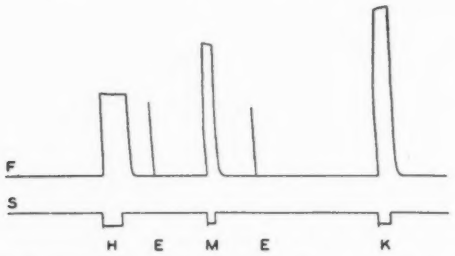

Fig. 1. Kymograph records of foot movements associated with a partly degenerated sciatic nerve (13 days) from the frog. The lower line, *S*, indicates periods of tetanizing stimulation, about one to two seconds each. The upper line, *F*, shows the resulting foot movements when the stimulus was applied to the nerve at the level of the hip, *H*, midway between hip and knee, *M*, and near the knee, *K*. When the electrodes were shifted the foot gave a momentary jerk, *E*.

tain which of these two conditions obtained, leg nerves from frogs after they had suffered a certain degree of degeneration were activated by identical electric stimuli applied at the three following regions: near the hip, midway between hip and knee, and just proximal to the knee. The state of the nerve was judged by noting the resulting movements of the foot. It was soon found that in the early stages of degeneration no difference in foot responses was to be observed to these types of stimulation. The foot moved as vigorously when the stimulus was applied at the hip as at the knee. This condition prevailed from the first day after the operation till the eleventh. On the twelfth day, however, differences first appeared and they were observed till the fifteenth day after which the condition of complete physiological degeneration intervened (table 1).

The character of the responses is clearly shown in kymograph records taken from nerve-leg preparations. Figure 1 shows the movements of

the foot from a frog at an early stage of nerve degeneration, *thirteen* days after the degnerative cut had been made, and with the sequence of the regions of stimulation as follows: hip, H; mid position between hip and knee, M; and knee, K. The lower line in the tracing records stimulation; a drop below the horizontal indicates the time during which a tetanizing electric stimulus was applied to the nerve, about one to two seconds. The procedure was as follows. The partly degenerated sciatic nerve was carefully dissected free from the hip to the knee and the nerve with a small bit of connective tissue at its proximal end for a handle was laid back on the leg of the frog. The electrodes were then placed under the

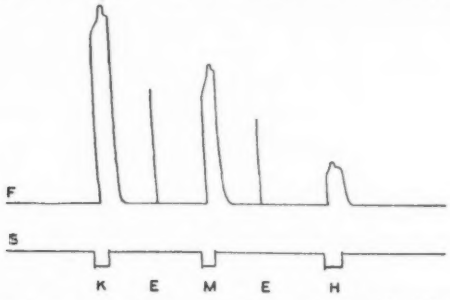

Fig. 2. Kymograph records of foot movements associated with a partly degenerated sciatic nerve (13 days) from the frog but in which the order of the stimulated regions is the reverse of that shown in figure 1. The lettering is the same as in figure 1.

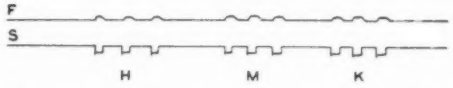

Fig. 3. Kymograph records of foot movements associated with a partly degenerated sciatic nerve (15 days). The lower line, S, represents periods of stimulation. The upper line shows foot movements. Three of these movements were excited at each position, hip, H; mid position, M, and near knee, K.

nerve in the region of the hip and a tetanizing stimulus applied. The foot movement elicited is recorded over H in the tracing. The electrodes were then shifted to a region on the nerve between the hip and the knee. This shift commonly called forth a momentary jerk of the foot which is recorded on the tracing at E. A second tetanizing stimulus was then given the nerve from this new position resulting in a foot movement shown at M. Again the electrodes were reset this time near the knee. This change also caused a momentary foot jerk after which the tetanizing stimulus at the third position called forth the foot movement shown at K. When the three foot responses thus induced are compared, it is evident that that from the stimulus applied at the hip is least, that from the

position between the hip and knee more and that from near the knee most extensive; in other words, the degree of degeneration of the nerve as indicated by the responses is fullest near the degenerative cut close to the hip and progressively less as the knee is approached.

In figure 2 a similar set of tests is recorded except that in this instance the first stimulus was applied near the knee, the next at the mid position and the last near the hip. Notwithstanding the reversed order of this application the foot responses remain as in the first instance: maximum from the knee, intermediate from the mid position, and minimum from the hip.

Figure 3 shows the movements of the foot activated through a nerve *fifteen* days after the degenerative cut had been made. Here stimulation of the nerve elicited from the foot musculature at best only a faint response. As can be seen from the kymograph record where three successive movements were excited at each of the three positions, near the hip they are minimum, at mid position intermediate, and near the knee maximum.

A comparison of the three sets of records shows at once a progressive series of tracing in which the stimulus when applied at the hip is less effective than at the mid position, and here less than near the knee. These conditions indicate that more fibers are functionally efficient at the knee than at the mid position and more there than at the hip. Put in another way, the axons of more motor units (to use a term recently introduced by Sir Charles Sherrington and his colleagues to indicate a single motor neurone with its hundred or so attached muscle fibers) are degenerate at the hip than at the mid position, where more are degenerate than at the knee. From this it follows that certain axons which at the hip are so far altered that they fail to conduct impulses may be fully able to act in this respect in the region of the knee. Such axons exhibit progressive degeneration and this degeneration is moving toward the periphery.

This type of progressive degeneration was found, as already stated, in the frog between the twelfth and the sixteenth day after the degenerative cut had been made. It was not always discoverable, however, in all the cut nerves of this period. Of sixteen nerves prepared over this interval eleven showed evidence of progressive degeneration but five did not. In these five I was unable to discover that one end of the nerve was capable of exciting a more vigorous response than the other. As the period is a relatively brief one, only some four or five days, it is possible that these five nerves had either passed this stage or had not yet arrived at it, but on this point I have no conclusive evidence. In no instance did I find a partly degenerated nerve in which the region of the hip called forth more vigorous foot responses than that of the knee. I therefore believe that the records reported point to the conclusion that from a physiological

standpoint frog nerve gives clear evidence of progressive degeneration. It is worthy of note that the period during which this nerve shows progressive physiological degeneration (the twelfth to the sixteenth day after the degenerative cut) agrees very well with the time when the progressive histological degeneration of the lateral-line nerve of the catfish is best seen (the thirteenth to the fifteenth day after the degenerative cut).

SUMMARY

1. Transected sciatic nerves of the frog may transmit nerve impulses without signs of physiological degeneration from the first to the sixth day, may show part physiological degeneration from the fifth to the sixteenth day, and complete degeneration from the sixteenth to the twentieth day.

2. Physiological evidence of *progressive* degeneration, as contrasted with *simultaneous* degeneration, occurs in the frog from the twelfth to the fifteenth day (18°C).

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NERVE CATALASE

FRANCIS O. SCHMITT AND ROYCE K. SKOW

From the Department of Zoology, Washington University, St. Louis

Received for publication July 18, 1933

Evidence has been offered by Zeile and Hellström (1930) and Kuhn, Hand and Florkin (1931) that catalase and peroxidase contain the heme group, and that, as in the case of Warburg's respiratory enzyme, it is this group which is catalytically active. These two enzymes, which are almost universally present in aerobic cells, are also stated to be of the same high order of activity as Warburg's oxygenase (Haldane, 1931; Kuhn, Hand and Florkin, 1931). Cyanide inhibits all three enzymes very completely in low concentrations. Little is known concerning the action of carbon monoxide on catalase and peroxidase.

From the application of the carbon monoxide inhibitor technique it appears that activation of oxygen is accomplished in nerve by a catalyst similar to Warburg's oxygenase. In this work on carbon monoxide poisoned nerve it has been assumed that the return of the spike and after-potential during illumination is caused by restoration of aerobic conditions in the nerve (Schmitt, 1930; Schmitt and Gasser, 1933).

The recent work on catalase and peroxidase, however, presents the possibility that these enzymes may play a rôle in nerve function hitherto unsuspected. According to the familiar thesis of Wieland, cyanide inhibits cell respiration and cell function by inactivation of catalase; the hydrogen peroxide which then accumulates, produces the toxic condition. Feng and Gerard (1930) claim that cyanide blocks the nerve action potential in split-sheath preparations very much faster than pure nitrogen. Perhaps this rapid failure is due to accumulation of hydrogen peroxide resulting from the cyanide inhibition of catalase. The question also arises whether catalase or peroxidase may be inactivated in simple asphyxia by accumulating metabolites. In this case, nerve failure may be due to inactivation of these enzymes rather than to exhaustion of oxidative reserve or of other precusor substances. We were particularly interested in the possibility that the photo-restoration of the action potential in carbon monoxide poisoned nerve may involve catalase or peroxidase as well as oxygenase. These possibilities assume considerable significance, not only because of the important rôle assigned to catalase and to peroxidase by the Wieland theory of biological oxidation, but also because of the

light which experiments on this subject might throw on the energetics of nerve conduction under anaerobic conditions.

It is generally agreed that the chief function of catalase is to prevent the accumulation of hydrogen peroxide in cells. There seems to be no such unanimity of opinion, however, regarding the function of peroxidase. Indeed, Elliott (1932) could demonstrate no action of milk peroxidase when tested on a variety of substrates such as fatty acids, fats, carbohydrates, amino acids, etc. Activity could be demonstrated only in the case of a few substances such as tryptophane, tyrosine and nitrites.

We have found it extremely difficult to test the reaction characteristics of peroxidase in frog nerve because of the large quantities of tissue required for the application of the standard tests. Micro-tests are difficult because of the necessity of isolating peroxidase from catalase and oxygenase. On the other hand, sensitive methods are already available for testing the activity of catalase, even in very small quantities of tissue such as nerve. Hence, in the experiments to be described below, we have confined our studies entirely to catalase.

METHOD. The activity of nerve catalase was determined manometrically as described by Fujita and Kodama (1931). The Warburg vessels were of about 15 cc. capacity, and were provided with an inset for alkali, a side arm to contain the H_2O_2, and a side outlet for gas perfusion. The customary technique was to place a bit of frog sciatic nerve 10 mm. long, weighing from 3 to 10 mgm. wet weight, in the vessel in about 0.4 cc. of phosphate Ringer solution. Five per cent KOH was placed in the inset to absorb any CO_2 that might be given off, and 0.2 cc. of H_2O_2 was placed in the side arm. The vessels were attached to simple or differential manometers, placed in the thermostat, and allowed to equilibrate for 15 minutes. The stopcocks were then closed and after a brief period, the H_2O_2 was tipped onto the nerves, resulting in the liberation of oxygen by the catalase. It was necessary to use only a small length of nerve so that the working range of the manometer might not be exceeded by the gas liberated.

Although in certain experiments it was expedient to work with intact nerve in the manner described, there are objections to the method. We found, for example, that the rate of the reaction depended to a large extent on the degree of injury of the nerve incident to the dissection. In test experiments in which one nerve was carefully dissected and the partner nerve deliberately injured it was found that the latter showed a rate of decomposition of H_2O_2 50 per cent higher than the former. The presence of the nerve sheath and of other diffusion barriers adds to the difficulty of obtaining strictly comparable rates either with partner nerves or with two strips from the same nerve. To avoid the possibility of contamination of the nerves with small particles of rust, which might also decompose H_2O_2,

the dissecting instruments were heavily chromium plated and were kept in oil when not in use. Despite all precautions, however, difficulty was encountered occasionally in obtaining comparable values for the catalase activity of similar nerves.

To obviate any error which might arise from this source, parallel experiments were performed upon nerve emulsion as the source of catalase. The nerve emulsion was prepared as follows: Six frog sciatics were placed in a mortar and ground to powder under liquid air.[1] As soon as the liquid air had evaporated, 10 cc. distilled water were added and allowed to freeze. The ice containing the nerve powder was then ground until a fairly homogeneous emulsion was obtained. To this were added 10 to 15 cc. of phosphate buffer, pH 7.6, and the emulsion centrifuged. The slightly opalescent supernatant fluid was separated from the precipitated débris and stored in the refrigerator. One cubic centimeter of such an emulsion liberates from 50 to 100 cu. mm. O_2 from 0.2 cc. of M/5 H_2O_2 in 30 minutes. Equal portions of emulsions so prepared gave figures for catalase activity which checked often as close as 5 per cent.

The hydrogen peroxide was of the best analytical reagent grade and was used as supplied or was distilled to avoid the presence of the acid preservative. In some of the later experiments specially purified 30 volume per cent peroxide was used which contained no preservative.[2] In all cases the final concentration was achieved by diluting the concentrated peroxide with phosphate buffer pH 7.6. According to Rona and Damboviceanu (1923), phosphate buffers tend to inhibit the action of catalase, but Fujita and Kodama (1931) failed to observe this effect. We found that freshly prepared phosphate buffer was without influence on the test, although considerable catalytic activity was evidenced after the buffer had stood for several days, even in the ice chest. Distilled peroxide and commercial peroxide were found to be equally satisfactory provided both were made up in phosphate buffer at the same pH. The concentration of the peroxide was determined by titration with $KMnO_4$ or manometrically by tipping MnO_2 or $KMnO_4$ onto the peroxide solution.

To determine the optimum pH for nerve catalase, emulsions were prepared and suspended in phosphate buffers of pH values ranging from 5.4 to 8.0, as measured with the glass electrode. Between pH 6.5 to 8.0 the activity of the catalase varied little; therefore, in the following experiments the pH was adjusted to 7.6 which corresponds to the pH at which nerve is usually kept for experimental purposes. Since catalase has been found

[1] It was found in preliminary experiments that the catalase activity of nerves subjected to liquid air treatment was not significantly different from that of partner nerves untreated.

[2] This reagent was supplied through the courtesy of Mr. J. L. Lanz of the Peroxide Chemical Company, of Saint Louis.

to show maximum activity at about 15°C., this temperature was used in most cases although a few of the later experiments were run at 20°C.

In work on catalase it is necessary, as Morgulis (1921) has shown, that conditions be adjusted so that the oxygen evolution shall be a direct function of the catalase concentration. Toward this end it is essential to determine the optimum concentration of hydrogen peroxide and to demonstrate that varying the quantity of catalase within reasonable limit does not affect the apparent activity of the catalase. The latter point is of less importance than the former in our experiments because of the unavoidable variability of the catalase activity from one emulsion to the next and because, in all experiments the same quantity of catalase was placed in each vessel. Nevertheless, control experiments were made in which it was demonstrated that variation in catalase concentration, holding the peroxide concentration constant, was, within certain limits, without effect on the apparent catalase activity.

TABLE 1

Effect of concentration of H_2O_2

Catalase kept constant = 0.1 cc. (obtained by emulsifying 20.5 mgm. nerve in 10 cc. PO_4 buffer).

CONCENTRATION OF H_2O_2	O_2 LIBERATED IN 30 MINUTES
M.	*cu. mm.*
0.146	15.5
0.176	16.0
0.220	15.7
0.293	14.7
0.440	15.7

It was found that the optimum peroxide concentration was from 0.2 to 0.4 molar. Under these conditions the oxygen liberated in thirty minutes was independent of the peroxide concentration (see table 1). In most of the experiments the peroxide concentration was 0.22 molar.

EXPERIMENTAL RESULTS. *1. Catalase activity of frog nerve.* Under aerobic conditions nerve seems to be well protected against hydrogen peroxide which might be formed by the union of activated hydrogen with molecular oxygen. Direct addition of H_2O_2 in low concentrations to nerve has little effect either on the respiration or on the action potential. The H_2O_2 is quickly decomposed and the normal curve of respiration is resumed (see fig. 4).

Although quantitative data are available concerning the catalase activity of the various tissues, we are unaware of any data on the catalase activity of peripheral nerve. Battelli and Stern (1910) quote the catalase index of amphibian and mammalian brain as 20 to 50, as compared with 3000 to

5000 for liver. Fujita and Kodama (1931), using the manometric technique, assign to brain the lowest catalase activity of any of the tissues studied by them. These authors made use of the term "catalase quotient" to express their results, the term being defined as the number of cubic millimeters of oxygen liberated in thirty minutes at 38°C. divided by the dry weight of the tissue in milligrams. They also stipulate that no values must be accepted where more than 50 per cent of the peroxide was decomposed. The catalase quotient of mammalian brain was found to be 50, as compared with 1700 for liver.

We find an average catalase quotient of 35 for nerves from summer frogs. This value is similar to that given by Fujita and Kodama for brain when

TABLE 2

Catalase activity of summer and winter nerves

DATE	TEMPERATURE	WET WEIGHT OF NERVE		O_2 EVOLUTION IN 30 MINUTES		CATALASE QUOTIENT*	
		Summer	Winter	Summer	Winter	Summer	Winter
	°C.	*mgm.*	*mgm.*	*cu. mm.*	*cu. mm.*		
2–13	15	4.0	4.0	29.1	19.6	36	25
2–14	15	6.0	6.0	35.6	21.9	30	19
2–16	15	3.6	3.6	24.2	15.2	33	21
3–18	20	9.2	9.2	84.5	92.3	46	50
3–20	18	9.4	9.8	54.1	46.1	29	23
				Average.....		35	28

* Catalase quotient $= \frac{\text{cu. mm. } O_2 \text{ evolved in 30 min.}}{\text{dry weight in mgm.}}$

It is assumed that dry weight is 20 per cent of wet weight.

allowance is made for the fact that our experiments were run at 15 to 18°C. instead of at 38°C.

It has long been known that blood is very rich in catalase; Fujita and Kodama list the catalase quotient of mammalian red cells as 6,000–12,000. Hence it became necessary to determine what error might arise by neglecting to correct for the catalase contained in the small amount of blood in the vascular system of the nerve. First, we determined the catalase content of frog nerves when the vascular system was freed of blood by perfusion with Ringer solution. No significant decrease was found as compared with unperfused control preparations. Furthermore, when the catalase content of frog blood was determined directly it was found that the value was too low to have any appreciable effect, considering the small amount present in a nerve. This is in agreement with the work of Krueger and Schuknecht (1929), who found that the relative catalase content of

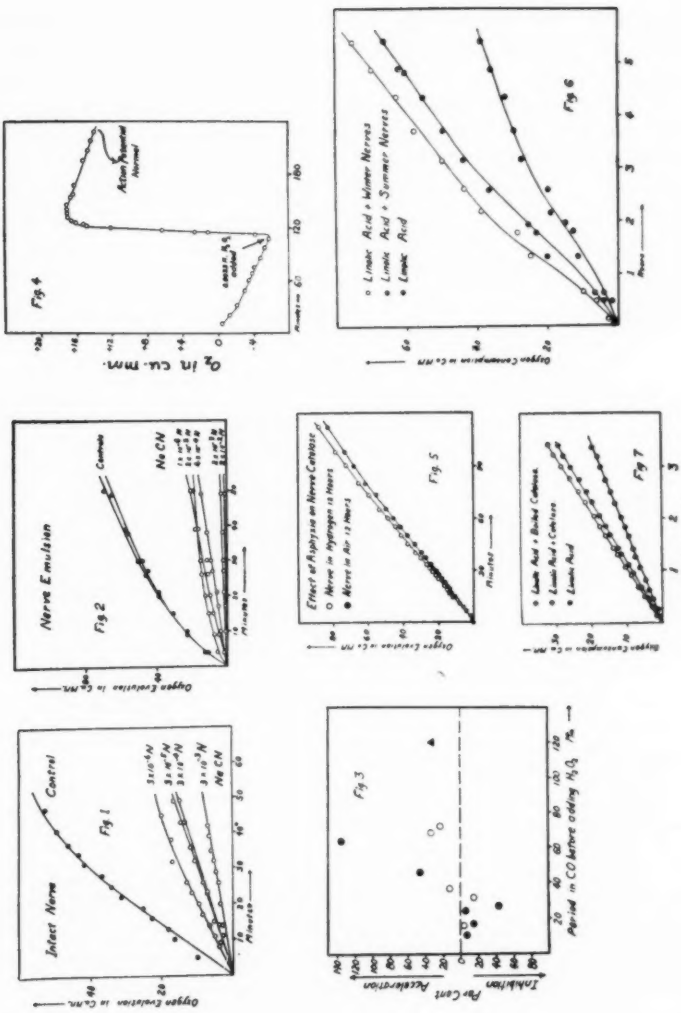
Fig 1
Fig 2
Fig 3
Fig 4
Fig 5
Fig 6
Fig 7
Inject Nerve
Control
Nerve Emulsion
Effect of Asphyxia on Nerve Catalase
Linolic Acid + Water Nerves
Linolic Acid + Summer Nerves
Linolic Acid
Linolic Acid + Boiled Catalase
Linolic Acid + Catalase
Linolic Acid
Oxygen Evolution in c.mm.
Oxygen Consumption in c.mm.
Minutes
Hours
Inhibition Per Cent
Acceleration
Period in CO before adding H2O2 Min.

Figs. 1 to 7

frog blood was less than 10 per cent, in the average, of that of mammalian blood.

Nerves from winter frogs which had been kept in a warm room for several weeks (summer frogs) show slightly higher catalase quotients than nerves from similar winter frogs kept in the ranarium at a temperature of 12 to 15°C. (see table 2).

2. The effect of NaCN and NaN_3 on nerve catalase. It has long been known that catalase is poisoned by traces of HCN (see Schönhein, 1868; Senter, 1904; Hata, 1909, and Zeile and Hellström, 1930). Similarly we

TABLE 3

The effect of carbon monoxide on catalase

PREPARATION	QUANTITY OF PREPARATION		TEMPERATURE	GAS MIXTURE		T^*	O_2 EVOLVED IN 30 MINUTES		INHIBITION
	Cont.	Exp.		Cont.	Exp.**		Cont.	Exp.	
			°C.		per cent CO	minutes	cu. mm.	cu. mm.	per cent
Intact nerve	6.0 mgm.	6.0 mgm.	15	Air	99.6	69	26.8	35.8	−33
Intact nerve	10.6 mgm.	10.6 mgm.	15	N_2	99.6	73	62.0	75.5	−22
Intact nerve	9.0 mgm.	9.0 mgm.	20	N_2	99.6	37	58.3	65.5	−12
Intact nerve	11.0 mgm.	11.0 mgm.	20	Air	94.2	17	136.0	132.1	3
Intact nerve	7.2 mgm.	7.2 mgm.	20	Air	90.2	32	62.7	53.0	15
Nerve emulsion	1.0 cc.	1.0 cc.	15	Air	99.6	120	77.5	103.4	−33
Extract***	0.2 cc.	0.2 cc.	20	Air	99	65	59.9	142.8	−138
Extract***	0.2 cc.	0.2 cc.	20	Air	98	18	118.8	102.2	14
Extract***	0.2 cc.	0.2 cc.	20	Air	92.7	25	189.2	177.7	6
Extract***	0.2 cc.	0.2 cc.	20	Air	94.6	46	87.0	126.8	−46
Extract***	0.2 cc.	0.2 cc.	20	Air	99	28	144.5	67.2	53
Extract***	0.2 cc.	0.2 cc.	20	Air	94.2	11	136.1	125.0	17

* Period in which the preparation was allowed to stand in CO before tipping the peroxide to make the test.

** The diluting gas is oxygen.

*** Extract of liver catalase made according to Zeile (1931) and diluted fifty times in phosphate buffer.

find that nerve catalase is blocked by cyanide in very low concentration both in intact nerve (fig. 1) and in nerve emulsion (fig. 2). Sodium azide, NaN_3, which acts like cyanide as an inhibitor of heme catalysts, also inhibits nerve catalase. With sodium azide in a concentration of M/5000, the inhibition is still 75 per cent complete.

3. The effect of CO on nerve catalase. Very little is known concerning the action of CO on catalase. Senter (1904) obtained no inhibition of blood catalase. Wieland (1925), in a brief statement, claims that liver catalase is inhibited 50 per cent by 0.00015 M CO. No data are given by Senter or Wieland as to the effect of illumination on this inhibition.

The experiments on CO contain a complicating factor not present in the cyanide experiments, namely, the time factor. In our earliest experiments we obtained inhibitions as high as 50 per cent with pure CO (Schmitt and Skow, 1933). Later experiments, however, failed to confirm this inhibitory effect; indeed, the CO accelerated the action of the catalase, in one instance as much as 138 per cent. It was found that the disagreement in results was due to variation in the time lapse between exposure to CO and the tipping of the peroxide. Figure 3, which is plotted from the data shown in table 3, illustrates this point. In this figure the circles, dots, and triangles represent, respectively, intact nerve, extract and nerve emulsion. In our earlier experiments this time lapse was short with the result that inhibition was obtained. When the catalase was left in pure CO for several hours the initial inhibition appears to have worn off and acceleration was observed. Similar results were obtained by Bredig and v. Berneck (1899) who found that palladium sol and other inorganic catalases undergo recovery of activity after prolonged exposure to CO.

We have never obtained any effect by illuminating catalase either in the presence of CO or in air.

4. The effect of anoxia on nerve catalase. Nerve contains a high percentage of unsaturated fatty acids which, in the course of oxidation, form unstable addition products with oxygen in the form of peroxides. Hydrolysis of these peroxides might be the source of hydrogen peroxide under anaerobic conditions. It is, therefore, of importance to determine whether the accumulating products of anoxic metabolism have an inhibitory influence on catalase. This possibility is emphasized by the relatively low concentration of catalase in nerve.

It will be seen from figure 5 that after 12 hours in H_2, nerve catalase activity remains unimpaired. Apparently lactic acid and other products of anaerobic metabolism are not destructive to catalase. From this it follows that it is impossible for H_2O_2 to accumulate in asphyxiated nerve.

5. Thermostable oxidation catalysts in nerve. We observed in connection with certain experiments on the mechanism of anaerobic nerve function that bits of dried nerve substance catalyze the autoxidation of unsaturated fatty acids. In these experiments the freshly dissected nerves were immersed in 95 per cent alcohol for one minute and allowed to dry on glass plates. They were then cut into small bits, weighed, and placed in Warburg vessels. One cubic centimeter of linolic acid was pipetted into the main chamber of each vessel and into each inset, 0.5 cc. of 5 per cent KOH. It will be seen from figure 6 that not only is the rate of oxygen uptake of the linolic acid accelerated over 70 per cent by the presence of the nerve substance, but that winter nerves seem somewhat more effective gram for gram than summer nerves.

Now it is well known from the work of Robinson (1924), Kuhn and

Meyer (1929) and Wright and Van Alstyne (1931) that heme compounds actively catalyze the oxidation of unsaturated fatty acids. This suggested the possibility that catalase in nerve may act not only as a peroxide-splitting catalyst but also as a heme catalyst of the oxidation of unsaturated fatty acids. A crude test of this point was made by studying the effect of a catalase extract on the oxidation of linolic acid. The extract was made from liver according to the direction of Zeile (1931) and was found to be extremely potent. One cubic centimeter of this extract added to 1 cc. of linolic acid caused a 40 to 50 per cent acceleration in the oxidation of the acid, whether the extract was boiled or unboiled (see fig. 7).

It cannot be concluded from these experiments that the thermostable substance in nerve, responsible for the catalysis of fatty acid oxidation, is the heme residue of catalase alone. Comparing a dilution of the extract which would have a catalytic effect on oxidation roughly equal that of a given weight of nerve, we found that the catalatic activity of this extract was still some twenty fold higher than that of the nerve substance. While this is but a crude approximation, it seems reasonable to expect that substances in nerve other than catalase may furnish heme residues which could be active in this regard. Although there is little or no cytochrome in frog nerve, other hematin compounds which might act as oxidation catalysts are doubtless present in quantities far more significant than the minute traces of these extremely active enzymes. We will refer to the possible rôle of these catalysts in anaerobic nerve function in another place.

SUMMARY

1. Frog nerve contains relatively little catalase, the catalase quotient being only 35. The catalase activity of summer nerves is somewhat higher than that of winter nerves.

2. Sodium cyanide and sodium azide, even in very dilute solutions, strongly inhibit the action of catalase both in intact nerve and in nerve emulsion.

3. Carbon monoxide may inhibit or accelerate the action of catalase, depending on the length of time which the material is exposed to the gas; brief exposures, up to about thirty minutes, produce inhibition, longer exposures produce acceleration. Light has no effect on the carbon monoxide treated enzyme. Catalase apparently plays no part in the photorestoration of the action potential in carbon monoxide poisoned nerve.

4. Catalase activity is unimpaired after asphyxiation of the nerve in hydrogen for extended periods.

5. Nerve contains thermostable substances which catalyze the oxidation of unsaturated fatty acids. Catalase, being a heme compound, acts

similarly but it is unlikely that the catalysis by nerve substance is due to catalase alone because the enzyme is present only in minute quantities.

The expenses of this investigation were defrayed by a Research Grant to Washington University by the Rockefeller Foundation.

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THE OUTPUT OF EPINEPHRIN FROM THE ADRENAL GLANDS DURING ANAPHYLACTIC SHOCK[1]

MILTON B. COHEN, J. A. RUDOLPH, P. WASSERMAN AND J. M. ROGOFF

From the Laboratory of Experimental Medicine, School of Medicine, Western Reserve University

Received for publication May 17, 1933

The use of epinephrin as a therapeutic agent in certain allergic conditions suggests a possible relationship of the adrenal glands in such phenomena. Increased epinephrin output from the adrenals, during anaphylactic shock, was reported by Houssay and Molinelli (1926) and by Tournade and Hermann (1927). We have investigated the epinephrin output from the adrenal glands by the quantitative method of assay employed extensively by Stewart and Rogoff (1917, 1919, 1923) and have been unable to demonstrate an increase due to anaphylactic shock. Our results are summarized here but, to save space, further details and discussion of methods are reserved for another article to be published in the Journal of Allergy (1933).

Our experiments were performed on dogs. Nine animals were sensitized with horse serum, 5 cc. on two or three successive or alternate days. The first dose was administered subcutaneously in all cases, the subsequent doses intravenously in some and subcutaneously in others. The shock experiments and determinations of epinephrin output were performed four to five weeks after administration of the sensitizing serum. In two animals intravenous injections of 5 cc. horse serum failed to produce shock, in all the others definite shock was obtained varying in intensity. The maximum fall in blood pressure observed was 90 mm. Hg the minimum 24 mm. The maximum initial pressure, before shock, was 140 mm., the minimum 74 mm. The maximum pressure observed during shock was 50 mm., the minimum 16 mm. The other characteristic phenomena (especially intestinal) of anaphylaxis in dogs were observed in all the animals, varying in degree with the intensity of the shock. Clotting of the blood was very much delayed in the shocked animals.

In one dog (no. 6) there was no evidence whatever of any effect produced by administration of the shock dose of horse serum. In another (no. 1) the injection of serum did not cause visible phenomena of shock. However, the blood flow from the adrenals was reduced to about one-third and the red blood cell count increased from 7,100,000 to 9,560,000. It is

[1] The expense of this investigation was shared by The Asthma, Hay Fever and Allergy Foundation.

possible, therefore, that there was a moderate anaphylactic reaction which did not manifest itself in a profound change in blood pressure. The pressure, already low (60 mm.), only fell to 50 mm. Hg. Whether or not the observed phenomena are interpreted as an anaphylactic reaction, there was no change in the epinephrin output from the adrenals. Dog 7 was pregnant and the uterus, with the fetuses, was removed before the collection of specimen I.

For convenience, the results of our experiments are presented in tabular form. No essential differences in the rates of epinephrin secretion, before and after induction of shock, occurred in any of the experiments.

TABLE 1

Epinephrin output before and after anaphylactic shock

NUMBER OF ANIMAL	SEX	BODY WEIGHT	BEFORE INTRAVENOUS INJECTION OF HORSE SERUM					AFTER INTRAVENOUS INJECTION OF HORSE SERUM					
			Blood pressure	Blood flow per minute	Epinephrin concentration	Epinephrin output		Blood pressure	Blood flow per minute	Shock	Epinephrin concentration	Epinephrin output	
						Per animal per minute	Per kgm. per minute					Per animal per minute	Per kgm. per minute
		kgm.	*mm.*	*gms.*		*mgm.*	*mgm.*	*mm.*	*gms.*			*mgm.*	*mgm.*
1	F.	12	60	7 4	1:3,000,000	0 0025	0 0002	50	2 3	−	1:1,000,000	0 0023	0 0002
2	M.	7 9	74	3 55	1:1,750,000	0 002	0 00025	50	2 6	+	1:1,000,000	0 0026	0 00032
3	M.	4 5	74	3 25	1:2,500,000	0 0013	0 00029	28	0 74	++	1:200,000	0 0037	0 00082
4	F.	14 2	114	12 8	1:5,000,000	0 0026	0 00018	30	2 0	++++	1:500,000	0 004	0 00028
5	M.	14 3	140	18 6	1:3,000,000	0 0062	0 00043	48	1 4	+++	1:250,000	0 0056	0 00039
								46	2 1		1:500,000	0 0042	0 0003
6	M.	16 1	96					100		−			
7	F.	9 1	78	4 2	1:3,000,000	0 0014	0 00015	20	0 32	+++	1:200,000	0 0015	0 00017
8	F.	9 1	80	22 2	1:5,000,000	0 0044	0 00049	16	7 3	++++	1:1,700,000	0 0043	0 00048
9	F.	5	110	5 1	1:4,000,000	0 0013	0 00026	32	0 8	++++	1:200,000	0 004	0 0008
								20	0 6		1:225,000	0 0027	0 00054

CONCLUSION

Anaphylactic shock, in dogs sensitized with horse serum, does not cause any significant alteration in the rate of epinephrin secretion from the adrenal glands.

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UTERINE BLEEDING OF MONKEYS IN RELATION TO NEURAL AND VASCULAR PROCESSES

II. Spinal-Cord Transection and the Oestrin-Level

G. van WAGENEN and S. ZUCKERMAN

From Yale University School of Medicine, New Haven, Conn.

Received for publication July 19, 1933

Uterine bleeding occurs from two to nine days after transection of the thoracic part of the spinal cord of normal monkeys (mangabeys and rhesus monkeys, eighth to twelfth thoracic segments, van Wagenen 1933). It did not occur in one animal (M.m. 41) in which transection was performed after bilateral gonadectomy, a fact which suggests that the ovaries are primarily involved in the chain of processes culminating in post-transection "menstruation." The following experiments were planned to investigate this possibility, and to throw some light on the mechanisms concerned in the phenomenon.

Castrated and immature animals. The observation on the castrate animal was first confirmed, uterine bleeding again failing to follow division of the spinal cord of a healthy, mature, gonadectomized rhesus monkey.

Experiment 1. M.m. 51, Rhesus monkey. Received October 18, 1932. The animal was gonadectomized on April 7, 1933. Uterine bleeding began three days after the operation and continued intermittently for fifteen days. The vaginal lavage was examined daily. The animal's spinal cord was divided on May 2, a few days after red blood cells had ceased to appear in the smear.

Operation. Body weight, 5,130 grams. Animal anesthetized with nembutal. Laminectomy of tenth thoracic vertebra, and cord transected between the eleventh and twelfth thoracic segments.

Post-operative notes. The vaginal lavage was repeatedly examined for four weeks, but no uterine bleeding was detected. The animal was used in a terminal experiment (reported below in the conclusion to this paper) and was sacrificed on the second day of post-theelin uterine bleeding.

Notes at autopsy. Body weight, 4,305 grams. Ulceration anterior to callosities and on lateral aspect of right ankle. Superficial veins greatly distended. All internal organs appeared healthy. Levels of transection verified.

Histological notes. Uterus: The uterus is small, presumably due to "castration atrophy." The outer fourth of the endometrium in the body of the uterus is completely disintegrated, and the greatest depth of the remaining healthy part of the mucosa is approximately 1.5 mm. The central cavity is filled with blood and fragments of endometrial tissue. A somewhat even line separates the healthy mucosa from the broken-down tissue; the latter looks as though it is about to be passed through the cavum uteri in large pieces. Glands are fairly numerous in the healthy

areas, moderately open, generally empty of secretion, and mostly straight. They reach to the limits of the stroma, which has an even line of junction with the myometrium. A few glands are branched in their deeper parts, and some are slightly coiled. The glandular epithelium is high columnar in type, with basal nuclei which show up as elongated compact bodies. Mitoses are uncommon. The stroma is tightly packed.

It is noteworthy that this uterus is not unduly vascular. Its general appearance suggests that the endometrial breakdown was a catastrophic event rather than a gradual process.

This negative result with castrate monkeys suggested that the bleeding will follow transection only if the uterus is under the influence of ovarian hormones. Experimental support for this hypothesis was found in the fact that bleeding did not occur when the operation was performed on a healthy immature rhesus monkey.

Experiment 2. M.m. 73, Rhesus monkey. Received May 4, 1933. Operated upon May 10, 1933.

Operation. Body weight, 2,490 grams. Animal anesthetized with nembutal. Laminectomy of seventh and eighth thoracic vertebra. Cord transected between eighth and ninth thoracic segments.

Post-operative notes. The vaginal lavage was examined repeatedly until May 23, but there was no sign of uterine bleeding. Instead of sacrificing the monkey immediately, we decided to give it a course of Progynon injections.[1] Three cubic centimeters were injected over eleven days, representing an amount stated to equal 6,000 rat units of oestrin. The sexual skin began swelling about the fifth day, and continued doing so until the end of the series of injections. This shows that the sexual skin, even though it has never shown signs of activity before, can respond to the presence of oestrin after spinal transection. An observation reported in the first part of this series of papers shows that the sexual skin of adult monkeys is also able to respond to theelin after spinal transection.

Injections were stopped on June 2, and the animal was sacrificed on June 9, but no uterine bleeding occurred between these two dates.

Notes at autopsy. Body weight, 2,520 grams. The only teeth of the permanent dentition that have erupted are the first molars and the eight incisors. Ulceration in neighbourhood of callosities, over the left greater trochanter, and on the dorsal aspect of the toes of both feet, which were very edematous. Ovaries small; the uterus both small and pale. The level of transection was verified.

Histological notes. Left ovary. Size after fixation 6 mm. × 4 mm. × 3 mm. There are about six follicles which have a diameter between 0.5 mm. and 1.0 mm., but only one contains an ovum which seems to be normal. The others appear to be in process of degeneration. Many smaller healthy follicles are present, and the number of primordial follicles does not seem to have been reduced as a result of the theelin injections to which the animal was subjected (Allen, 1928, is of opinion that theelin reduces the number of these follicles in immature rhesus monkeys). There are a considerable number of atretic follicles, and apparently all phases of the process of atresia are represented.

Right ovary. It was noticed at autopsy that this ovary was cystic. Size after fixation 8.0 mm. × 5.0 mm. × 3.5 mm. There are about eight follicles which in

[1] Kindly supplied by Dr. Gregory Stragnell of the Medical Research Division of the Schering Corporation, New York City.

diameter measure between 0.5 and 1.0 mm., and there is one which measures 4 mm. × 2.5 mm. × 2.5 mm. This cystic follicle is situated more towards the lateral than the medial pole of the ovary, and in greater part consists of a single layer of irregularly cuboidal cells surrounded by theca which cannot with any certainty be divided into two layers. The follicular origin of this cyst is proved by the appearance of its medial pole, although its lateral end, which is in close relation both with the hilum and the Fallopian tube, could possibly be mistaken for one of those columnar celled "cysts" so commonly seen in ovaries in this situation (one is present in the left ovary of this animal). The cyst distorts the general topography of the ovary, pressing on other follicles, but apart from this, and a lesser degree of atresia, the ovary is more or less similar in histological appearance to its fellow. It is impossible to discover from the sections the nature of the contents of the cyst, but in a few places highly eosinophilic material can be seen passing into it through its lining epithelium.

Uterus. The uterus is small, but the simple mucosa is deeper than is usual in immature rhesus monkeys, and in places extends for 2 mm. The latter fact can be attributed to the theelin injections the animal received previous to its death (cf. Allen, 1928). The line of junction of mucosa and myometrium is even, and the glands, which are few, straight, and open, extend almost to the muscle coat. In greater part the surface epithelium consists of tall columnar cells, with nuclei which are somewhat vesicular, and usually oval in shape. They are irregularly disposed in the cells. The glandular cells are taller, and their nuclei are more evenly disposed—the majority towards the middle zone of the cells. Both the surface and glandular epithelium are very vacuolated, and in many places their free margins are covered with droplets of secretion. The cavum uteri and the glands nevertheless appear empty. In places the surface epithelium is heaped, and in one area new epithelium has grown in to cover a breach caused by a small extravasation of blood which has lifted the old epithelium (still present) from the stroma. This one area is the only sign of uterine bleeding in the prepared sections, but there are many patches of epithelial degeneration whose appearance suggests rupture of cell membranes following pressure of intra-cellular material. Mitoses are numerous, both in the surface cells and in the glands. There is also a conspicuous leucocytic infiltration of the cervix, and many polymorphs can be seen in process of passing through the cervical epithelium. The stroma is moderately and uniformly edematous. Its cells are of various shapes, the majority being elongated. Mitoses are present, but are not so frequent as in the epithelial constituents of the endometrium.

Castrated animals receiving theelin. All except one of the seven positive experiments on normal animals, previously reported, were performed in the earlier half of the cycle, at a time when it is generally believed that the uterus is under the influence of only the follicular hormone of the ovary. Experiments were therefore planned to discover whether uterine bleeding follows cord section primarily as an effect of the operation upon an oestrin-sensitized uterus, or secondarily, as a result of a drop in the oestrin level following a change occurring in the ovaries after transection. Section of the cord in castrated animals receiving theelin injections was chosen as the economical way of throwing light on this problem.

Experiment 3. Cf. 8, Sooty mangabey. Received November 19, 1931. The animal was gonadectomized on January 22, 1932, and in the interval between this operation and the present experiment had received four courses of theelin injections,

to the last of which it had reacted by uterine bleeding beginning on February 27, 1933, ten days after the cessation of injections.

A fifth course of theelin injections[2] was begun on March 8, and continued for twenty-two days, a total of 7,250 rat units being given. On separate days three theelin suppositories, each stated to contain 50 rat units, were also inserted. The amount of resultant sexual-skin swelling was not as great as that produced during the previous course of theelin injections, when fewer rat units were administered. The animal's spinal cord was divided on March 24, the seventeenth day of injection.

Operation. Animal anesthetized with 5 per cent nembutal (0.8 cc. per kgm. of body weight), and ether. Laminectomy of seventh and eighth thoracic vertebrae. Cord divided between radices of eighth and ninth thoracic nerves.

Post-operative notes. The animal was kept alive for six days after the operation, the theelin injections being continued without interruption. The sexual skin fluctuated in size, and on the second post-operative day was smaller than at any time during the pre-operative period. A very few red blood corpuscles were occasionally seen in preparations of the vaginal lavage, but there was no definite sign of uterine bleeding.

The monkey failed to recover its strength after this operation, and was unable to lift its trunk with its arms. It was therefore sacrificed on March 30.

Notes at autopsy. Body weight, 7,560 grams. Some fairly well organized blood clots were present in the intradural space in the higher levels of the cord. Otherwise the organs of this animal appeared normal. The uterus was somewhat congested and of fair size. The transection level was verified.

Histological notes. Uterus. The greatest depth of endometrium in the body of the uterus is 3 mm., and the mucosa meets the myometrium in an even line. The glands are fairly numerous, and the majority are straight tubules which extend to the deepest parts of the stroma, where some of them branch. A few are coiled, but none is widely open. In some places the epithelium becomes progressively taller as it is traced from the surface to the deeper parts of the glands. In the latter region the nuclei are more evenly, and more basally, disposed, and they are also less vesicular than the nuclei of the surface. The epithelial cells are considerably vacuolated, especially in the superficial zone of the mucosa, where their appearance suggests that the nuclei have moved towards the free margins of the cells owing to the accumulation of vacuoles. Some of these highly vacuolated cells contain eosinophilic "inclusion bodies," but these are confined to the cytoplasm and do not encroach on the nucleus. There is very little secretion, and mitoses, though present, are not numerous. Many leucocytes are in process of migration through the surface epithelium, and numbers have accumulated in the superficial zone of the stroma, which is less tightly packed than the deeper part. Most of the stromal cells in this somewhat edematous superficial zone have round vesicular nuclei. In the deeper part of the endometrium the stromal nuclei are mostly spindle shaped.

Experiment 4. M.m. 25, Rhesus monkey. Received January 16, 1932. The animal gave birth on June 18, 1932, and was gonadectomized on February 21, 1933, two days after the beginning of a menstrual period which lasted three days. Uterine bleeding recurred six days after the operation. Daily theelin injections were begun on March 8 and were continued for twenty-five days, a total of 7,000 rat units being injected. The spinal cord was divided on March 21, the fourteenth day of injection.

Operation. Body weight, 6,025 grams. Animal anesthetized with nembutal. Laminectomy of tenth thoracic vertebra, and cord divided at tenth thoracic segment.

[2] Kindly supplied by the Parke Davis Co., through the courtesy of Dr. Oliver Kamm.

Post-operative notes. Uterine bleeding did not occur during the eleven post-operative days during which theelin injections were continued, but bleeding began seven days after the cessation of injections, and continued for three. Preparations of the vaginal lavage were studied daily, and on some occasions a few red blood cells were seen, but only once were there more than one or two to an occasional high power field. In such numbers they are a frequent finding in the vaginal lavage of normal animals during the interval between menstrual periods. (We have been unable to discover any periodicity in the appearance of the cells. They have also occasionally been seen in the lavage from castrate animals.)

M.m. 25 remained in very good condition during the month following this operation, and we therefore decided to divide her spinal cord a second time. The operation was performed on April 24 in the usual way, and the cord transected between the radicles of the eighth and ninth thoracic nerves. Theelin injections were not given either before or after this second transection. Vaginal smears were studied daily. No uterine bleeding occurred at any time during the sixteen days immediately following this transection. At this point we decided to sacrifice the animal, but as a terminal operation her spinal cord was divided for a third time. The final section was made at the level of the second thoracic (spinal cord) segment. The animal was killed two days later, on May 12.

The monkey had a fairly bright sexual-skin color even at the time of her death. This is noteworthy insofar as she had been a castrate for two and a half months, and had not received theelin for six weeks previous to death.

Notes at autopsy. There were no superficial ulcers. The hind limbs were fixed in a flexor position. The uterus was of good size. The levels of the three transections were verified.

Histological notes. Uterus. The uterus is very small ("castration atrophy"), and the line of junction of endometrium and myometrium is fairly regular. The greatest depth of mucosa in the body of the uterus is about 1 mm. The lining of the central cavity is fairly even and unbroken, and consists of low columnar epithelium with big irregular nuclei filling the greater part of the cell bodies. The cavum uteri contains secretion. There are a moderate number of glands, all of which are straight and open. Few contain secretion. The glandular epithelium is taller than that of the surface, and its nuclei are more elongated. They fill from about $\frac{1}{2}$ to $\frac{1}{3}$ of the inner parts of the cells. No mitotic figures were seen either in the glandular or surface epithelium. The stroma is more tightly packed towards the cavum uteri, and the stromal cells show a considerable change in character as they are traced from the surface to the myometrium, where they begin to take on the appearance of myo- and fibro-blasts. The endometrial vessels are dilated.

Discussion. The fact that uterine bleeding did not occur in either of the two castrate monkeys (M.m. 41 and 51) or the immature monkey (M.m. 73) after spinal cord transection clearly suggests that such bleeding will take place only when the uterus is being sensitized by ovarian secretions, in particular oestrin. The possibility that luteal hormones are also concerned has not yet been tested.

The way in which oestrin is implicated is indicated by the experiments with the mangabey C.f. 8 and the macaque M.m. 25. The experiment in which the mangabey was used should be regarded as inconclusive owing to the necessity that arose for sacrificing the animal so soon after the division of her spinal cord. The experiment with M.m. 25 is, on the other

hand, fairly clear in its implications. Since uterine bleeding did not occur, in spite of the transection, while theelin (oestrin) was being injected (injections being continued for a day longer than the longest latent period yet observed between transection and the "menstruation" it induces), the experiment indicates that the uterine bleeding which follows division of the spinal cord can hardly be a direct effect of the operation upon an oestrin-sensitized uterus. Consequently it may be supposed that the bleeding which follows transection in normal monkeys is at least in great part due to a drop in the level of the ovarian secretion of the hormone, an event which on the basis of spinal cord physiology may be tentatively supposed to result from vascular changes occurring below the level of transection. The experiments imply that the uterine bleeding which follows the operation in normal monkeys would be delayed or inhibited if the pre-operative level of oestrin were experimentally maintained.

It is of course possible that some significance attaches to the occurrence, noted above, of an occasional erythrocyte in the vaginal lavage of C.f. 8 and M.m. 25 after the first transection of the cord. These cells may represent a direct effect of the operation upon the sensitized uterus. This possibility of a direct effect, which also applies to the post-operative fluctuations in size of the sexual skin of the mangabey, needs further experimental investigation.

M.m. 51, the gonadectomized macaque whose spinal cord had been transected, was used in a terminal operation in which the conditions were made to simulate what we suppose to be the major changes occurring in the reproductive organs of the normal animal after cord transection. The animal was given a course of daily theelin injections for thirteen days, a total of 3,600 rat units being injected. On May 29, the day the injections were stopped, her spinal cord was divided a second time, on this occasion between the radicles of the seventh and eighth thoracic nerves. A drop in the oestrin level was thus made to coincide with cord transection. Uterine bleeding began on the tenth day after the operation. The animal was sacrificed on the second day of bleeding.

SUMMARY

1. Transection of the spinal cords of castrated monkeys does not induce uterine bleeding (three cases, M.m. 41, M.m. 51, M.m. 25, second transection).

Transection of the spinal cord of an immature monkey did not induce uterine bleeding. The previously inactive sexual skin of this animal responded to the injection of theelin after this operation.

These experiments therefore suggest that uterine bleeding follows transection only when the uterus is under ovarian influence. In the light of

the data presented in the first part of this series of papers it follows *a priori* that the hormone oestrin is in particular concerned.

2. Transection of the cord of two castrate monkeys receiving theelin injections did not induce uterine bleeding. In the one case injections were continued for a period longer than the longest latent interval yet observed between cord transection and uterine bleeding in normal monkeys. Bleeding began ten days after injections were stopped.

These experiments thus imply that post-transection uterine bleeding does not primarily represent an effect of the operation upon an oestrin-sensitized uterus. Consequently it may be supposed that the bleeding largely reflects a fall in the uterine oestrin-level.

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THE RÔLE OF THE PORTAL SYSTEM IN THE REGULATION OF CIRCULATING BLOOD VOLUME

GEORGE M. ROBERTS AND LATHAN A. CRANDALL, JR.

From the Department of Medicine and the Department of Physiology and Pharmacology, Northwestern University Medical School

Received for publication July 18, 1933

Miller and Poindexter (1932) have recently shown that the rapid intravenous injection of large quantities of saline does not change the blood volume of the normal dog, as measured by the dye method. A marked discrepancy was noted between the volume of circulating blood and the dilution of the blood as indicated by changes in red count, hematocrit, and blood protein. It seemed unlikely that the injected saline could be removed from the vascular system as rapidly as it was introduced; in fact the persistence of blood dilution showed that such rapid removal does not take place unless one assumes that red cells and protein are lost from the vessels along with the excess fluid. Using the Welcher method, they found that the total blood volume was considerably increased. It occurred to us that the finding of a normal blood volume within a few minutes after the intravenous injection of large quantities of saline could be explained by a storage of the diluted blood in certain tissues in such a manner that while it still remained in the vascular system, it was in effect removed from the circulation.

The mechanism by which such a storage of blood in the vascular system may be effected has been the subject of a number of investigations, which are reviewed by Chanutin, Smith and Mendel (1924) and Krogh (1929). The latter writer states that "the portal system, comprising the capillaries, venules, and veins of the stomach, intestine, and spleen together with the portal trunk itself, acts as a variable reservoir of blood in virtue of the double set of resistances with which it is provided." Even in view of the evidence to support the assumption that an abrupt increase in blood volume may be thus compensated for by withdrawal of a corresponding quantity of blood from the circulation, it is somewhat difficult to conceive of a relatively large amount of blood being so isolated in the portal system that it is not at all mixed with the injected dye during the course of several minutes. Such a conception indeed requires a rigid distinction between the "circulating" blood volume and the "total" blood volume.

If storage of blood in the portal system is a mechanism for keeping the

"circulating" blood volume constant, then intravenous injections in animals deprived of this mechanism should be followed by an increased blood volume as measured by the dye method. We have selected the Eck fistula as a means of preventing any possible accumulations of blood in the portal vessels, and have compared the effects of intravenous injections in normal and Eck fistula dogs.

Intravenous fluid in normal dogs. We have repeated and confirmed the experiments of Miller and Poindexter in which large amounts of isotonic saline were given rapidly by vein; we have also administered 5 per cent dextrose solution in the same way. Blood volumes were determined by the dye method, using brilliant vital red (Hooper et al., 1920). In our hands, the estimation of blood volumes at short intervals has not given consistent results; we have preferred not to make a second determination until the dye has completely disappeared from the blood stream, which requires slightly less than three days. In determining the effect of intravenous fluid upon blood volume, we have therefore established the normal of the particular animal by at least two determinations made at three-day intervals. The observed variation in the blood volume of the normal uninjected animal has been less than 5 per cent, provided the weight is constant. In animals that show marked changes in body weight, as is the case with Eck fistula dogs, the absolute blood volume may vary considerably but the amount of blood per kilo body weight has never fluctuated more than 10 per cent and usually does not vary more than 5 per cent.

To test the effect of intravenous fluid, the animal was given 0.9 per cent NaCl or 5 per cent dextrose warmed to 40°C. at the rate of 100 cc. per minute. The amounts given ranged from 50 to 105 per cent of the animal's normal blood volume. Control blood samples were taken 2 to 10 minutes after completing the injection of fluid, the dye was immediately injected, and the final blood sample was removed 5 minutes after the injection of the dye. The time between injection of fluid and determination of blood volume is taken as the interval from completion of fluid administration to the injection of dye. Where repeated experiments on the effect of intravenous fluid were made on the same animal, the normal blood volume was again determined between each such experiment, the usual three-day period intervening between each dye injection. Lindhard (1926) has observed that after dye has been frequently injected, it is removed more rapidly from the blood; he believes that not more than 7 blood volume determinations can be made with accuracy. We have therefore not measured the blood volume more than 7 times in any one animal. All the studies were made on unanesthetized animals that were trained to lie quietly throughout the procedures.

The effect of intravenous fluid on the blood volume was determined in 9 normal dogs, saline being given to 6 and dextrose to 3. The results obtained are shown in table 1. The observations of Miller and Poindexter

(1932) that intravenous saline does not increase the apparent blood volume are confirmed, and it is shown that the same is true of dextrose. At the same time, the plasma volume is definitely increased, again confirming these authors. This means that if the information obtained by the dye method is trustworthy, an amount of diluted blood equal in volume to the amount of fluid injected is not available for mixture with the injected dye. The plasma volume increases very materially at the expense of the cell volume. It is difficult for us to conceive of such a rapid loss of cells from the vascular system, especially since it has been shown repeatedly that after such injections of fluid, the cell volume returns almost to normal in about 3 hours. The volume of the cells that disappear commonly amounts to about 200 cc. in a 10 kilo dog. There is no evidence that even under the provocation

TABLE 1

Blood volume after intravenous fluid in normal dogs

DOG	AMOUNT ISOTONIC SALINE OR DEXTROSE (EXPRESSED AS PER CENT OF NORMAL BLOOD VOLUME)	MINUTES BETWEEN END OF FLUID INJECTION AND INJECTION OF DYE	INCREASE IN BLOOD VOLUME	INCREASE IN PLASMA VOLUME
			per cent	*per cent*
C1	50 (saline)	9	−10	18
C2	50 (saline)	2	0	31
C3	71 (saline)	2	8	33
C4	65 (saline)	3	5	36
C5	75 (saline)	2	5	42
C6	79 (saline)	3	7	35
C7	73 (dextrose)	3	−3	15
C8	68 (dextrose)	2	6	39
C9	78 (dextrose)	3	−2	24

of a severe hydremic plethora such a volume of cells can abruptly leave the vessels and then reappear within 3 hours. Still assuming the validity of the dye method, one is forced to the conclusion that somewhere within the vascular system there is a storage of blood, or possibly of cells alone.

There remains the possibility that under the conditions of these experiments, the dye method for blood volume determination does not give an accurate figure for the amount of circulating blood. If this were the case, one would expect that the apparent blood volume after intravenous fluid would vary widely, and would not consistently agree with the pre-fluid determinations. If the dye method were actually in error under these conditions, it would be expected that the error would be due to a loss of dye from the blood under the influence of the hydremic plethora. But such a loss of dye would give figures for the blood volume that would be too large, rather than the readings actually found. We have attempted to throw further light on what actually takes place by first injecting dye into

a normal animal and then immediately giving a quantity of saline almost equal to the animal's blood volume. If storage of diluted blood does take place, and if dye does not leave the vessels, the concentration of the dye under these conditions should permit an estimation of the total amount of blood remaining in the vascular system since the dye will be stored along with the diluted blood. Furthermore, such calculations of total blood volume should agree reasonably well with the total blood volume as calculated from the known normal cell volume and the hematocrit by the equation:

$$BV = \frac{CV}{\text{Hematocrit}} \qquad \begin{matrix}(CV = \text{cell volume}) \\ (BV = \text{blood volume})\end{matrix}$$

The results of such an experiment are given in table 2. It will be noted that the expected increase in blood volume occurs. About 400 to 500 cc. of fluid appear to have been lost from the blood during the injection and before

TABLE 2

Injection of dye followed by intravenous fluid. Normal blood volume of dog—1,270 cc.

TIME	PROCEDURE	TOTAL BLOOD VOLUME (CALCULATED FROM DYE)	HEMATOCRIT	BLOOD VOLUME (CALCULATED FROM HEMATOCRIT)
8:28	Normal blood sample		50.5	
8:29	Dye injected			
8:29–8:33	Gave 1 liter saline			
8:34	Blood sample	1,850	32.6	1,945
8:54	Blood sample	1,808	38.2	1,660
9:14	Blood sample	1,782	40.2	1,580

the first sample was taken, but in spite of this, the blood volume as calculated from the concentration of dye agrees well with that calculated from change in hematocrit. In fact the change in dye concentration as a result of the injection is seen to be slightly less than the change in percentage of cells, although the difference is only 5 per cent and probably within the limits of error. In the 21 and 41 minute samples, the blood volume calculated from the hematocrit drops more rapidly than that calculated from dye concentration; this could be due either to a loss of dye from the vessels or to a release of red cells from such storage depots as the spleen, and is very likely due to both.

Since it appears unlikely that any of the factors that might invalidate the use of the dye method after intravenous fluid would act to produce an actual *increase* in dye concentration of sufficient magnitude to return the calculated blood volume to normal, and since the mechanisms for a storage of blood in the vascular system itself are known and have been discussed, a demonstration of an increase of blood volume after intravenous fluid in

animals whose storage mechanisms have been abolished should be a rather conclusive demonstration that such storage may take place. The need for indubitable evidence arises from the difficulty of conceiving of such a complete isolation of large amounts of blood. The stored blood must be so held apart from the circulating blood that no dye is mixed with it.

TABLE 3

The effect of transfusion on blood volume

DOG	AMOUNT OF BLOOD GIVEN (EXPRESSED AS PER CENT OF NORMAL BLOOD VOLUME)	MINUTES BETWEEN END OF TRANSFUSION AND DYE INJECTION	INCREASE IN BLOOD VOLUME	INCREASE IN PLASMA VOLUME
			per cent	*per cent*
C1	18	4	−5	0
C2	37	3	4	12
C3	33 (slight shock)	3	−22	−12

TABLE 4

Blood volume after intravenous fluid in dogs with Eck fistulae

DOG	POST-OPERATIVE WEEK	AMOUNT ISOTONIC SALINE OR DEXTROSE (EXPRESSED AS PER CENT OF NORMAL BLOOD VOLUME)	MINUTES BETWEEN END OF INJECTION AND DYE ADMINISTRATION	INCREASE IN BLOOD VOLUME	INCREASE IN PLASMA VOLUME
				per cent	*per cen*
E29	46	81 (saline)	2	75	121
E36	8	72 (saline)	6	39	51
E37	8	88 (saline)	3	32	46
		88 (saline)	2	37	69
		72 (dextrose)	2	29	65
E39	3	71 (saline)	2	−1	28
		69 (saline)	3	0	25
E41	3	71 (saline)	2	15	56
		54 (dextrose)	1½	24	36
E48	42	102 (saline)	4	24	66
E49	38	90 (saline)	3	23	34
E52	21	98 (saline)	3	18	28
E53	4	105 (saline)	3	30	51
E54	4	85 (saline)	3	37	63
		86 (dextrose)	3	34	68

It seemed worth while to attempt to increase the blood volume of normal animals by blood transfusion. Storage in the portal system should occur as readily after the administration of whole blood as after saline, although the rate of fluid loss from the vascular system would presumably be less. Table 3 gives the results of 3 such experiments. The difficulty of interpreting these data is illustrated by dog C_3 in which slight shock was evinced by nausea and a single attempt to vomit, although the animal was willing to

run about. Although all the animals were cross matched prior to the transfusion, which was carried out by the Percy (whole blood, paraffined glass tube) technique, the shock in this animal seems to have resulted in a vascular dilatation and consequently a blood volume below even its normal value. Because of the possible difficulty of recognizing changes in the caliber of the vessels that might be produced by incompatible blood even in the absence of external symptoms, this series of animals was not extended. One should note, however, that there was no appreciable increase in blood volume following transfusion.

Intravenous fluid in Eck fistula dogs. The injections of saline and glucose were repeated in Eck fistula dogs, using the same technique as in the normal animals. Ten dogs were employed, the experiments being performed from two weeks to ten months after the operation.

The results in these animals are given in table 4, and it will be noted that in every case except one (E_{39}) the intravenous injection of fluid has produced a distinct increase in the blood volume, and as would be expected, a more marked increase in plasma volume. In most instances, the increase accounts for 30 to 60 per cent of the injected fluid. If the return of the hematocrit toward normal may be taken as an index of the rate at which the excess fluid is eliminated from the vascular system, this rate is extremely rapid. Our figures on percentage cells before and after fluid indicate that some 40 to 60 per cent of the injected fluid is commonly lost during the injection and in the next 2 or 3 minutes thereafter. Unfortunately such figures cannot be considered as more than approximations, since it is possible that an extrusion of red cells from the spleen introduces an error that causes the rate of disappearance of fluid to appear too high. Smith and Mendel (1920) used the red cell concentration as an index of blood volume in rabbits after the injection of a volume of saline equal to the animal's blood volume, and found that more than 50 per cent of the injected fluid left the vascular system within three minutes.

If we are able to accept such changes as a crude indication of the amount of fluid lost, then it seems probable that storage in the portal system is able to account for the greater part of the added fluid that remains within the vascular system. We have no explanation of the failure to increase the blood volume in dog E_{39}. This animal was autopsied and the Eck fistula appeared to be adequate in size and functioning well.

It should be stated that dogs E_{29} and E_{36} were transfused with amounts of blood equal to 32 and 52 per cent of their normal blood volumes, respectively. The observed increases in blood volume were 12 per cent in the first case and 31 per cent in the second.

It is also interesting to note that the amount of blood per kilo body weight is frequently higher in the Eck fistula dog than in the normal. The animals as a rule lose weight for a few weeks after the operation, and we

have observed that although the blood volume falls, the decrease is not as rapid as that in body weight. In 15 normal animals, the average number of cubic centimeters of blood per kilo was 86, the maximum 110, minimum 70. In the 10 dogs with Eck fistula, the average was 93, maximum 115, minimum 76.

Effect of intravenous fluid after splenectomy or splanchnotomy. Because of the known ability of the spleen to store red cells, it seemed possible that this organ might share the ability of the portal system to compensate for abrupt increases in blood volume. Three dogs were splenectomized and fluid was given intravenously according to the usual technique. In none of these animals was there any appreciable increase in blood volume, they behaved like normal dogs. The spleen was also removed from dog E_{33}, in which the response to intravenous fluid had not been changed by Eck fistula. Splenectomy had no effect in this animal. We have confirmed the report of Abderhalden and Roske (1927) that splenectomy causes a decrease in the blood volume. In our 3 animals, the decrease one week after the operation varied from 22 to 33 per cent. Abderhalden and Roske did not apparently note any return of the blood volume to normal; they explain the phenomenon on the basis that the spleen removes some dye from the circulation. In our animals, the blood volume had returned to normal or above by the third post-operative week, which suggests that the decrease is not secondary to the taking-up of dye. No blood was lost at operation except that which was present in the spleen, and no body weight changes were observed post-operatively. In the splenectomized Eck fistula dog, the decrease in blood volume amounted to 20 per cent and had not returned to normal in 2 months.

In 2 dogs, the splanchnics were cut aseptically to see whether removal of the vasoconstrictor nerves from the splanchnic area would reproduce the picture of the Eck fistula dog as regards ability to handle excess fluid. In these animals after rapid intravenous injections amounting to 80 and 74 per cent of the normal blood volumes, increases of 33 and 23 per cent respectively were noted in the blood volumes while the plasma volumes increased 45 and 42 per cent. This indicates that the storage of fluid in the portal system is under the control of a nervous mechanism.

Discussion. Since our experiments produced conditions that can never occur in the normal animal, i.e., an abrupt addition of 50 to 100 per cent to the volume of the vascular system, it is a matter of conjecture whether this mechanism for the maintenance of a constant volume of circulating blood plays an important rôle under normal conditions. However, the rate of absorption of fluid from the gastro-intestinal tract is known to be very rapid, and it may be that this means of maintaining homeostasis is useful when large volumes of water are being absorbed from the intestines. It appears even more likely that storage in the portal system comes into play when for any reason there is a tendency for the mass of the circulating blood to shift

into the splanchnic area, as in a peripheral vasoconstriction. Simonds and Brandes (1927) have found that mechanical obstruction of the hepatic veins of the dog produces a fall in arterial blood pressure similar to that which occurs in anaphylactic shock, and that the injection of serum to which the dogs are sensitive produces no further fall of blood pressure when the hepatic veins are occluded. Nor does occlusion of the hepatic veins at the height of the blood pressure fall in anaphylactic shock produce any further decrease in blood pressure. One is led to the conclusion that storage of blood in the portal area must occur, in the dog at least, during the anaphylactic reaction. The anatomical considerations that bear upon this question are discussed by Simonds and Arey (1920). Barcroft (1925) has said: "Blood volume should be regarded as a physiologic variable which is adjusted to the work required of it and to the size of the 'bed' which it occupies." Without detracting from the validity of this statement, it might be added that under many conditions it is the "bed" that is the variable, and that mechanisms for maintaining the circulating blood volume constant are highly developed.

We cannot refrain from emphasizing the uselessness of depending upon variations in cellular composition or blood protein as an indication of variations in circulating blood volume. We see no other way of interpreting our results except as a mechanism for homeostasis applicable to the circulating blood volume. This requires a rigid distinction between that amount of blood in the vascular system which is being propelled by the heart and is performing the functions of the blood, and a further quantity of noncirculating blood which may be highly variable and which is held at least partly in the portal system. Variations in the concentration of various blood constituents may reflect with some accuracy changes in the total quantity of blood contained in the vessels. The amount of circulating blood appears to be measurable only by direct methods.

SUMMARY

The observation of previous investigators, that the blood volume as determined by the dye method remains constant after the intravenous injection of large quantities of saline, is confirmed. This is also true following intravenous glucose and transfusions of whole blood. This finding is analyzed in the light of similar determinations on Eck fistula dogs, splenectomized dogs, and dogs with section of both splanchnic nerves. Intravenous fluid produces an increase in the blood volume (dye method) in the Eck fistula and splanchnotomized animals, but not in those that have been splenectomized. It is shown that if dye is given prior to the injection of saline, the dilution of the dye is comparable to that of the red cells. The action of the portal system as a variable reservoir for blood is discussed in connection with these results, and *the conclusion is reached that the portal*

system is capable of storing a large quantity of blood in such a manner that it is not available for dilution of the dye. Such storage appears to account for the greater part of the excess fluid that does not promptly leave the vessels after intravenous injection. The necessary distinction between total and circulating blood volumes is emphasized.

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CARBOHYDRATE METABOLISM, RESPIRATION AND CIRCULATION IN ANIMALS WITH BASAL METABOLISM HEIGHTENED BY DINITROPHENOL

V. E. HALL, J. FIELD 2ND, M. SAHYUN, W. C. CUTTING AND M. L. TAINTER[1]

From the Departments of Physiology and of Pharmacology and the Division of Neuropsychiatry, Stanford University School of Medicine, California

Received for publication July 17, 1933

Of the substances now known to augment the oxidative metabolism of animal organisms by direct action upon the cells, thyroxin and epinephrine are the most prominent. As agents for the experimental study of the response to increased metabolic turnover they have certain disadvantages: thyroxin, the slow onset of its effect; epinephrine, the complication of sympathetic stimulation. Through the work of Cutting and Tainter (1932, 1933), another substance, 1-2-4 dinitrophenol, possessing the required effect, has become available. With doses between 3 and 20 mgm. per kilogram no response was seen that was not clearly attributable to an acceleration of intracellular oxidation, while even prolonged administration produced neither loss of weight, impairment of liver function, abnormalities in blood or urine composition, nor histologically demonstrable lesions of important viscera. Further, we have found no increase in urinary nitrogen excretion. Similar findings were reported independently by Magne, Mayer and Plantefol (1931, 1932). We have, therefore, in the use of this substance, a means for comparing the bodily manifestations in heightened basal metabolism with those in the normal animal in a manner not hitherto possible.

METHODS. Six experiments were performed, dogs anesthetized with pentobarbital being used. Oxygen consumption, respiratory rate and tidal air were recorded continuously by means of a Sanborn closed-circuit metabolism apparatus. In certain experiments, the water reservoir was filled with ice water to prevent undue rise in the temperature of the respired gas which otherwise appeared and impeded heat dissipation. Blood glucose was determined by the Folin-Wu method as modified by Sahyun (1931); blood and muscle lactates by that of Friedmann, Cotonio and Shaffer; and liver and muscle glycogen by that of Sahyun. In certain experiments the

[1] Supported in part by the Rockefeller Fluid Research Grant of the Stanford University School of Medicine.

cardiac output was determined by application of the Fick principle, the oxygen content of blood samples drawn under oil from the carotid artery and right atrium being obtained by the manometric method of Van Slyke and Neill (1924).

After a control period during which samples of blood, liver and muscle were taken and initial values of respiratory and circulatory variables established, a dose of 10 or 20 mgm. per kilogram of dinitrophenol was injected intramuscularly, enough sodium carbonate being added to form the soluble sodium salt. A period averaging 66 minutes was then allowed to elapse for the full effect of the drug to develop, whereupon tissue samples were again taken and the experiment discontinued.

General effects. Within two minutes after the injection, the dog begins to manifest signs of a marked increase in metabolism; the breathing becomes deeper and more rapid, the oxygen consumption increases, the body temperature rises and the arterial pressure increases. These changes become progressively greater until the animal is hot to the touch, the breathing becoming extremely hyperpneic but regular. In some cases in which there is a fatal outcome, the breathing finally becomes irregular, slow and dyspneic and the arterial pressure begins to fall. At this time the oxygen consumption shows a decline. Within ten minutes at the most after the onset of these disturbances the animal dies with a body temperature of from 42 to 46°C. As noted by Tainter and Cutting (1933), a rigor of the muscles often sets in before death, presumably due to the excessive body temperature.

The body temperature rise was definitely noticeable in about five minutes and continued upward without interruption. The rate of rise averaged 0.24°C. per minute with 20 mgm. per kilo., and 0.1°C. per minute with 10 mgm. per kilo. The maximum rise was 9.4°, from an initial of 37.0° to 46.4°. However, in this case the expired air temperature rose to 32°. When the temperature of the expired air was prevented from rising by ice water, the maximum rise of body temperature was 4.5°.

The oxygen consumption of the dogs was invariably increased to a very marked extent, as is shown by the data in table 1. The increase in oxygen consumption was always clearly demonstrable within the two minute period immediately following the injection, thus indicating very rapid absorption and distribution of the drug. In experiments which were permitted to run until a maximum of oxygen consumption had been attained, the maxima were reached with a dose of 20 mgm. per kilo. at 19 minutes and with 10 mgm. per kilo. at 52 and 55 minutes after injection. The rise to the maximum was fairly regular.

Interrelations of hyperthermia and increased oxygen consumption. The fact that the oxygen consumption begins to increase and may often be at least doubled before the body temperature rises perceptibly shows clearly

that the increased oxidation is responsible for the hyperthermia. That the accelerated metabolism produces enough heat to account quantitatively for the rise in body temperature is shown by the following calculation, based on the data of one experiment. Total *excess* oxygen consumed, 5.48 l.; heat equivalent, at 4.77 Cal. per liter of oxygen, 26.14 Cal.; specific heat of body, 0.83 (Pembrey, 1898); body weight, 15.2 kgm.; "predicted" rise in body temperature, 5.7°C.; observed rise in body temperature, 4.4°C. All experiments with dose of 10 mgm. per kilo gave results similar to those in the example cited. The discrepancy is attributable to an increased rate of heat dissipation at the higher body temperature.

It is of interest to know to what extent the increased rate of oxygen consumption, at the moment when it has reached its maximum, can be accounted for in the manner described by van't Hoff's rule by the increase in

TABLE 1

Oxygen consumption

EXPERIMENT NUMBER	BODY WEIGHT	INITIAL OXYGEN CONSUMPTION	MAXIMUM OXYGEN CONSUMPTION	TOTAL INCREASE IN OXYGEN CONSUMPTION	HYPERTHERMIA INCREASE IN OXYGEN CONSUMPTION	TEMPERATURE RISE
Dose, 20 mgm. per kilo						
	kgm.	*cc./min.*	*cc./min.*	*per cent*	*per cent*	*°C.*
1	11.35	33	336	918	49	4.2
2	15.3	112	1538	1270	76	6.0
Dose, 10 mgm. per kilo						
3	11.35	73	465	537	85	6.5
4	15.2	105	434	313	40	3.6
5	5.5	51	213	317	50	4.3
6	17.7	144	521	262	20	1.9

body temperature. Employing the value for the Q_{10} of oxygen consumption of 2.57, which we have recalculated from data of DuBois (1921), and the conventional formula for this temperature coefficient, we have calculated the per cent increases attributable to the hyperthermia. As may be seen in table 1, these increases constitute a relatively small part of the total rise in metabolism observed. The remainder, that portion of the rise attributable to the action of the drug *per se* at that moment, is clearly related in magnitude to the dose employed.

Changes in the chemical composition of blood and other tissues. Table 3 contains the results of analysis of blood, muscle and liver. In skeletal muscle, there is invariably a marked fall in the glycogen content, averaging 0.215 per cent and 0.135 per cent with the 20 and 10 mgm. per kilo doses. This decrease in muscle carbohydrate is in part offset by an increase in

muscle lactate of 0.076 per cent and 0.026 per cent for the same two doses. The carbohydrate actually disappearing amounted to 0.139 per cent and 0.081 per cent, this material doubtless serving as a part of the fuel of the accelerated metabolism.

In consonance with the small rise in muscle lactate, the blood lactate changes were irregular and usually not striking, although moderate rises were common. The moderate changes shown by the lactates indicate that no serious anoxemia had developed in the animals at the time of sampling. However, in experiment 3 a sample of muscle taken at the end of the experiment, when the animal had gone into rigor at a body temperature of 46.4°C. showed a lactate concentration of 187 mgm. per cent, a rise of 62 mgm. per cent over the value of 12 minutes before. Blood lactates rose from 42 to 64 mgm. per cent in the same interval. These results indicate that there was a terminal anoxemia.

The liver glycogen was markedly decreased in all cases in which it was determined. With the 10 mgm. per kilo dose the average fall was 1.44 per cent. The interpretation of this fact is facilitated by the finding of a consistent increase in blood glucose concentration amounting on the average to 70 mgm. per cent (in experiments with 10 mgm. per kilo), a finding which suggests that liver glycogenolysis was occurring actively.

The concentrations of blood urea nitrogen and blood amino-acid nitrogen showed small increases, averaging 2.45 and 1.2 mgm. per cent respectively, in the two experiments in which they were determined.

Supplementary data were obtained in a series of four cats anesthetized with amytal or urethane, in which the alkali reserve was determined by Van Slyke's method, and the blood pH by the method of Hastings and Sendroy (1924). It was found that as long as the respiratory volume was maintained so that the blood appeared well oxygenated, there was no appreciable change in the CO_2 combining power of the plasma from the normal average of 39.8 vols. per cent nor in the pH from 7.37. Near the end of the experiment, when the respiration became irregular and shallower, the blood became more acid, and the CO_2 combining power decreased to an average value of 22.1 vol. per cent. At death the blood pH was about 6.95. These results indicate that as long as the respiration ventilated the blood adequately the acid-base balance and pH remained fairly constant and no acidosis developed. These results are consistent with our results on the dogs which showed that lactates did not materially increase until death approached.

Relation of the carbohydrate loss to oxygen consumption. In order to determine the nature of the fuel utilized during the period of accelerated metabolism, we have made quantitative comparisons between the oxygen equivalent of the carbohydrate disappearing and the observed oxygen consumption, employing the methods of calculation described by Hall and

Sahyun (1933). A sample balance sheet follows: Experiment 4. Weight of dog, 15.2 kgm., muscle, 5.55 kgm., liver, 0.460 kgm., blood volume, 1.68 l.

Muscle: glycogen	−0.079%		
lactate	+0.012		
total	−0.067%	−3.715 gm.	
Liver glycogen	−2.362	−10.868	
Blood lactate	−0.014	−0.235	
Total glucose	+0.043	+3.268	
Total carbohydrate change		−11.550 gm.	
Oxygen equivalent of carbohydrate	8.63 l.		Actual oxygen consumption
Oxygen unaccounted for	14.00 l.		22.63 l.
	22.63 l.		22.63 l.

The results of the experiments with the 10 mgm. per kilo dose are summarized in table 2. On the assumption that no protein was oxidized, the

TABLE 2

Chemical changes in tissues

EXPERIMENT NUMBER	MUSCLE				LIVER		BLOOD				DERIVATIVE CALCULATIONS			
	Glycogen		Lactate		Glycogen		Glucose		Lactates		Total ebh. lost	Oxygen equivalent	Actual oxygen consumed	Oxygen oxidizing ebh.
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final				
	per cent	*per cent*	*mgm. per cent*	*mgm. per cent*	*per cent*	*per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*grams*	*liters*	*liters*	*per cent*
1	0.465	0.232	68	144					19	95				
2	0.417	0.219	79	155					33	34				
3	0.210	0.070	84	125			116	155	23	42	7.667	5.73	23.89	24.0
4	0.300	0.225	78	90	4.95	2.70	78	121	38	24	11.550	8.63	22.63	38.1
5	0.356	0.165			2.73	1.78	133	210	53	46	4.124	3.08	8.64	35.7
6	1.175	1.040			2.23	1.12	103	172	26	94	0.385	0.29	11.57	2.5

R.Q. in the above experiments would be 0.77, 0.81, 0.81 and 0.71 respectively, the average being 0.78. In the series of control experiments the R.Q. estimated from the same type of data and assumptions averaged 0.80. Accordingly, there is nothing in our results on anesthetized dogs which would indicate that the accelerating effect of dinitrophenol on oxidative metabolism caused any notable change in the proportion of nutrients serving as fuel for such metabolism.

Inasmuch as the methods of estimating R.Q. employed above are open to criticism on the ground of the indirectness of approach and the assumptions invoked, recourse was had to a supplementary observation on rats, which show a response to dinitrophenol similar to that of dogs (Cutting and

Tainter, 1932). In 19 experiments, the oxygen consumption and carbon dioxide production were measured in a closed circuit metabolism apparatus. After two control periods of 15 minutes each, the rats were injected with 10 mgm. per kilo of dinitrophenol, and the metabolism was observed for three or more subsequent periods. In the control periods, the average oxygen consumption was 1069 cc. per kilo per hour, and the carbon dioxide production 997 cc., giving a R.Q. of 0.93. With each 15 minute period after dinitrophenol, the metabolism increased until a peak was reached in the third period with an oxygen consumption of 1787 cc. and carbon dioxide output of 1409 cc., the R.Q. being 0.78. In some other rats, it was determined that the metabolism returned to normal in from five to seven hours.

Although the control R.Q. of the rats differed distinctly from that of the dogs, it is notable that the R.Q. during the action of the drug in rats and dogs agrees exactly. The persistence of these relatively low R.Q. values is in agreement with the fact that, although there occurs, during the action of dinitrophenol, a marked decrease in the carbohydrate content of the body, this will account for considerably less than half of the oxygen consumed. The preponderant rôle, as fuel of the oxidative processes, must then be played by fats, by proteins, or by a mixture of these nutrients. In this we disagree with Magne, Mayer and Plantefol (1932), who, observing large decreases in liver and muscle glycogen, together with increases in blood sugar, but neglecting any comparison with oxygen consumption, concluded that "les oxydations augmentées sous l'action du dinitrophénol portent d'abord et surtout sur les sucres."

In our experiments, no evidence concerning the partition between fat and protein of the non-carbohydrate fuel of metabolism was obtained. Magne, Mayer and Plantefol (1932) report that, in dogs fed on a meat diet of constant weight dinitrophenol administration did not increase the urinary nitrogen excretion. These findings have been confirmed for the cat by Hall and Wooliever (unpublished). Accordingly, the presumption is that the principal nutrient oxidized is fat.

Circulatory response. From table 3 it is apparent that the transport of oxygen to the tissues, augmented under the action of dinitrophenol, is carried out by a distinct increase in the minute volume of circulation, to approximately two and one-half times the initial value on the average. The arterio-venous difference increased in two cases, but fell in a third.

Respiratory response. As is shown in table 4, there occurs, as a response to the increased metabolism during the action of the drug, both an increase in the tidal air and the respiratory rate, with consequent large increase in the minute volume of respiration. Of these two factors, the increase of rate is the more important. It is noteworthy that the respiratory stimulation is not consistently greater with doses of 20 mgm. per kilo than with doses of 10 mgm. per kilo.

A comparison of the proportionate increase in respiration and oxygen consumption shows that the respiratory response is in all cases much greater than would be necessary to preserve the proportion of oxygen taken out of the inspired air by the blood under pre-injection conditions. In other words, the respiratory stimulation is proportionately greater than is the

TABLE 3

Circulatory response

EXPERIMENT	BEFORE INJECTION				AFTER INJECTION			
	Arterial oxygen	Venous oxygen	A-V O_2 Diff.	M. V.	Arterial oxygen	Venous oxygen	A-V O_2 Diff.	M. V.
	volume per cent	*volume per cent*	*volume per cent*	*liters per minute*	*volume per cent*	*volume per cent*	*volume per cent*	*liters per minute*
4	20.00	8.74	11.26	0.94	15.30	5.54	9.76	4.47
5	19.50	13.14	6.36	0.79	21.04	4.26	16.78	1.27
6	21.34	16.04	5.30	2.75	14.14	5.77	8.37	4.11

TABLE 4

Summary of respiratory response

EXPERIMENT	BEFORE INJECTION			AFTER INJECTION			RATIO $\frac{\text{AFTER INJECTION}}{\text{BEFORE INJECTION}}$		
	Tidal air	Rate per min.	M. V.	Tidal air	Rate per min.	M. V.	Tidal air	Rate	M. V.
					20 mgm./kgm.				
	cc.		*liters*	*cc.*		*liters*			
1	64	7	0.38	264	35	9.24	4.12	5.00	15.06
2	88	34	2.81	584	84	49.06	6.56	2.47	17.53
					Averages.....		5.34	3.73	16.30
					10 mgm./kgm.				
3	144	5	0.68	176	81	14.26	1.22	16.02	20.96
4	96	25	2.34	440	86	37.84	4.58	3.44	16.14
6	344	7	2.61	1408	16	21.54	4.09	2.29	8.26
					Averages.....		3.30	7.31	15.12

metabolic stimulation. On the other hand, there is a fairly good linear relation between the increase in respiration and the increase in body temperature. This fact suggests that the high body temperature, acting either directly through the blood on the respiratory center, or through the intermediary of temperature receptors, is an important factor in the respiratory stimulation observed.

Discussion. As has been emphasized in the work of Cutting and Tainter (1932), all of the disturbances provoked by the administration of dinitrophenol are attributable to a single primary action, that of accelerating the rate of oxidative metabolism. The magnitude of this acceleration may approach values attained physiologically only under conditions of vigorous muscular exercise and far exceed those resulting from administration of the hormones normally concerned with the regulation of metabolism, thyroxin and epinephrine.

As to the nature of the "excess" oxidative metabolism evoked by this drug, the evidence at present available may be summarized as follows: 1, neither anoxemia nor acidosis developes as long as the respiratory and circulatory mechanisms keep pace with the oxygen usage; 2, no considerable accumulation of lactates occurs in the muscles—a fact indicating that the "recovery" processes are not falling behind in handling any load placed upon them; and 3, the R.Q. of the total and "*excess*" metabolism are the same; and 4, no catabolism of tissue protein occurs, as is shown by the absence of any increase in urinary nitrogen excretion. These findings fail to reveal any difference between the "excess" metabolism of dinitrophenol and that occurring normally.

For use in metabolic experiments on mammals, anesthetized or unanesthetized, we recommend a dosage range of 3 to 15 mgm. per kilogram of body weight depending upon the degree of metabolic stimulation desired. Animals receiving such doses will ordinarily survive, provided extraneous pathogenic factors are absent. Higher doses will yield greater degrees of metabolic stimulation but the animals usually die. The drug may be administered subcutaneously or intramuscularly, but is also active when given by mouth.

SUMMARY

1. Administration of dinitrophenol, by accelerating tissue metabolism, provokes an increase in oxygen consumption which may amount to over ten times the resting rate.

2. The body temperature rise which follows the increase in oxygen consumption is secondary to the acceleration in metabolism.

3. During the action of the drug, pronounced decreases occur in liver and muscle glycogen, while blood sugar and lactates and muscle lactates tend to rise. The total carbohydrate disappearing, however, accounts for less than half of the oxygen consumption. The principal fuel of the accelerated metabolism must therefore be other than carbohydrate.

4. The increased oxygen consumed is carried to the tissues by means both of an increased minute volume of circulating blood and of a greater arteriovenous oxygen difference.

5. Both respiratory rate and tidal air are increased. The respiratory

stimulation is out of proportion to increase in oxygen consumption, and is correlated rather with the degree of hyperthermia developed.

6. With fatal doses, a decrease in respiration and oxygen consumption occurs shortly before death. At this time lactates rise and blood CO_2 combining power and pH fall, indicating the development of a pre-mortal anoxemia and acidosis. However, with non-fatal doses, none of the above phenomena appear.

7. Dinitrophenol is well adapted to the experimental study of the various responses of organisms to conditions of accelerated tissue metabolism.

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ON THE MECHANISM OF ERECTIO.

V. E. HENDERSON AND M. H. ROEPKE

From the Department of Pharmacology, University of Toronto

Received for publication July 27, 1933

In the course of our studies (Henderson and Roepke, 1933) of the local hormonal mechanism of parasympathetic stimulation, we were able to show than an acetylcholine-like substance (AC) was produced on stimulation of the chorda tympani, even after the administration of such amounts of atropine as entirely prevented secretion. Indeed, the amount of AC liberated seemed as great as when secretion occurred. The AC so liberated, diffusing from gland endings to arterial muscles, could well account for the vasodilatation produced by chorda stimulation, which is known not to be depressed by atropine. Also, we found that after atropine the amount of acetylcholine injected intra-arterially had only to be increased some 4 to 5 times to produce an equal vasodilatation. The question as to whether there were vasodilator fibres in the chorda and whether their stimulation produced AC, could not be resolved in this case. Consequently we were led to study the vasodilator fibres to the penis, in the hope that there the issue would not be confused by AC produced from other nerve endings. In the course of our work it became necessary to study certain other aspects of the phenomenon of erection, and these will also be reported. The literature will only be considered in connection with the experiments and comments thereon.

The technique employed was in general as follows: The penis was dissected free of skin and the prepuce cut and clamped or inserted into a plethysmograph, so that all venous return from the prepuce and glans penis was forced to pass back by the deep dorsal veins. The penis was then reflected caudally. One, or in some cases both deep dorsal veins were cannulated and if blood flow were to be recorded, united to a collection bottle system. The system employed differed somewhat from that usually described, and may be of value to other workers. A very short glass cannula was inserted into the vein. A short (2 to 3 cm.) rubber junction led the blood by an appropriately curved glass tube to the lower outlet of an aspirating bottle filled with 25 per cent magnesium sulphate solution. The top of the bottle was closed by a stopper through which passed a tube leading almost to the bottom. The outer end of the tube was curved downwards till its lower end was some 3 to 5 cm. lower than the vein. Thus there was a

slight negative pressure in the system and in the vein. To prevent clotting in the connections to the bottle, a slow stream of a saturated solution of sodium bicarbonate containing a little oxalate passed from an elevated aspirating bottle into the vein cannula through a fine hypodermic needle shoved through the rubber junction.

The nerves stimulated to cause dilatation were the fine fibres passing horizontally in the fold of the peritoneum stretching from the rectum to the prostate and membranous urethra, as suggested by Martin and Tainter (1923) and others.

In the perfusion experiments, a cannula was inserted into the lower end of the aorta and all branches, save those from the visceral branches of the hypogastric (the nomenclature is that of Ellenberger and Baum) known in human anatomy as the internal pudendal, were tied, so that the perfusion fluid passed only to the prostate, lower rectum in part, the urethra and penis, though some escaped doubtless, through arterial anastomoses if the pressure was high into the hemorrhoidal system. Perfusion was carried out by a Dale-Shuster pump with oxygenated Fleisch solution containing 1 part of physostigmine per million.

The chief erectile tissue occurs in the corpora cavernosa, the corpus spongiosum and the glans penis. Of these, the corpora cavernosa are probably the most important for erection, but all dilate on nerve stimulation. The arrangement of the venous outflow makes it almost impossible to collect clear perfusion fluid from all these sources, especially from the corpora cavernosa. The minute anatomy, arterial venous and nervous for the dog, is perhaps not fully known, but seems to parallel that described by Kiss (1921) and Testut (1931) for man. Apparently each corpus cavernosa receives one or two arterial branches which enter it posteriorly and one of which traverses its length, giving off helical branches. The connective tissue of the corpus sheath also receives fine twigs from the deep dorsal artery of the penis. Some of the venous blood from the corpus also passes out posteriorly and enters the venous anastomoses, known as the plexus of Santorini and the hemorrhoidal plexus. Consequently, not all of the perfusion fluid from the corpora was collected by us. The main arterial supply for the corpus spongiosum enters posteriorly, but some blood is derived from the deep dorsal arteries of the penis and evidently a considerable part of its venous return passes into the deep dorsal veins, as clamping the dorsal arteries close to the gland penis only cut the flow from the dorsal vein in our experiments about one-half. For example, in one experiment, we observed a decrease from 9 drops to $5\frac{1}{2}$ drops in ten seconds, and after stimulation of the dilator nerves, again only approximately one-half. The glans penis which is supplied by the dorsal arteries, then, in our experiments must have been contributing somewhat less than half the blood or perfusion fluid collected, the corpus spongiosum and the corpora cavernosa the rest.

On stimulating the dilator nerve, the blood flow increased from both dorsal veins as there is free anastomosis in the glans and corpus spongiosum. There was always a definite latent period varying from 20 to 30 seconds with a weak faradic stimulus barely felt in the tongue, to some 5 seconds with a very strong stimulus. The flow increased from one drop of blood in 2 to 4 seconds, to a steady stream, i.e., over 4 drops per second. Taking measured quantities, the increase was from 12 to 80 cc. per minute in one case where the flow was large. With weak stimuli the maximum rate of flow would be reached in some 20 to 30 seconds, and fell to normal in 15 seconds after stimulation ceased. After a maximal 20 second stimulation, the flow declined to normal in 60 to 90 seconds.

The intravenous administration of physostigmine increased the flow, decreased the threshold for nerve stimulation and increased the time taken for the flow to decline to normal. For example, in experiment 5.6.33, dog

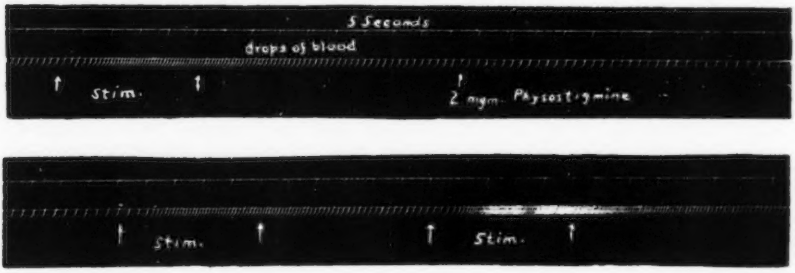

Fig. 1. The tracing is continuous. All stimulations of same length and intensity, coil 8 cm., frequency 5 per second. The next stimulation, one minute later, produced a stream.

25 kgm., the nerve was stimulated in each case for 20 seconds, with coil at 21 cm., 6 extra drops of blood; at 19, a stream declining to normal in 100 seconds. Then 3 mgm. physostigmine, coil at 21, extra drops 71; at 23, extra drops 12; coil 19, stream, back to normal in over 150 seconds. The same may be seen in figure 1. Where time and blood flow alone are shown from a dog of 26 kgm., stimuli are of constant strength, coil 8, with a low frequency of 5 per second. The tracing is continuous. As the physostigmine concentration in the tissue increased, the effect of stimulation became greater. The flow decreased more slowly to normal, and indeed another stimulation, one minute later, caused a stream which did not come back to normal for minutes. These effects of physostigmine strongly suggest an AC mechanism.

On perfusing, the flow was more rapid than for a corresponding arterial pressure, probably on account of the lower viscosity. It was, however,

increased by stimulating the dilator nerves, but not so greatly as the blood flow. In some cases the increase was only about twice. We had similar experiences when perfusing the salivary gland. Seven experiments were carried out. In the last six of these, the perfusion pressure was greatly reduced, so that the perfusion was very slow, 1.5 cc. per minute in the last two. In only the last experiment did the perfusion fluid, when diluted to be isotonic with frog's blood, show any evidence of an acetylcholine effect on the perfused frog's heart, Straub method. In this last case, a highly reactive frog's heart showed a depression of contractility, equivalent roughly to a concentration of 1:320 millions of acetylcholine. In such a weak dilution, and owing to the presence of an augmentor substance always present in such perfusion fluid, the assay is not very accurate. The cardiac effect was abolished by atropine. As it was thought that with a low pressure there might be difficulty in washing out any AC, a higher pressure and more rapid flow after a period of stimulation was tried, but no AC-like substance was obtained.

Further, control fluid and that from a period of stimulation, were taken down to dryness, taken up by alcohol and chloroform and an extract made. This then was acetylated. The acetylcholine present on assay was calculated on the basis of the amount of fluid originally obtained. Unfortunately such perfusion fluids tend to become progressively less blood tinged. In experiment 28.5.33, perfusion was begun at 12:45. One hundred and twenty-five cubic centimeters control fluid collected from 12:51 to 5, contained choline when acetylated equivalent to 1:60 millions. From 1:02 to 6 during stimulation, 130 cc. containing equivalent of 1:115 millions. From 1:07 to 11, control 55 cc., equalling 1:160 millions. Such experiments do not suggest any increase in the choline content of the perfusion fluid during stimulation.

Consequently, we can present no direct evidence that the local hormonal mechanism of dilatation is due to an AC mechanism, though the reaction to physostigmine certainly suggests that this is the case. Further, we have found in experiments not yet reported in extenso, that even from the urinary bladder the perfusion fluid may only contain the equivalent of 1:160 millions acetylcholine during stimulation, and it may be that very low concentrations are adequate to cause the reaction of smooth muscle and that AC cannot be so readily washed out as from the heart or salivary gland.

The injection of acetylcholine into the stump of the parietal branch of the hypogastric artery, when all but the above mentioned branches of the visceral branch were tied so that the injection passed via the visceral branch to the penis, caused a dilatation of the vessels. This effect was also increased by physostigmine. For example, experiment 5.6.33, dog 23 kgm. 1 cc. of 1:1,600,000 acetylcholine produced 2 extra drops of blood; 1 cc.

of 1:800,000 produced 9. After 3 mgm. of physostigmine intravenously, a repetition of these injections produced 35 and 50 extra drops respectively.

Atropine, 10 mgm. intravenously, greatly decreases the effect of arterial injection of acetylcholine, while decreasing but slightly or not all the effect of nerve stimulation. For example, in one experiment 1 cc. of acetylcholine 1:50,000 injected in 10 seconds, produced 14 extra drops, and 1:25,000 a stream of at least 20 drops in 10 seconds. After atropine 1 cc. of 1:2,000 produced no change in flow. That nerve stimulation was little affected by even large doses of atropine was observed by Langley and Anderson (p. 107, 1896), and by v. Anrep and Cybulski, and confirmed by us. It does not seem probable that the decreased effect of acetylcholine injected intra-arterially after atropine in the case of the penis can be due to the explanation advanced by Dale (1914) namely that after atropine the acetylcholine produced a nicotine-like stimulation of vasoconstrictor ganglia, since Langley and Anderson (p. 135, 1896), have shown that the sympathetic neurons for the vasoconstrictors of the penis lie largely in the sacral ganglia, and would hence be reached only after the acetylcholine had passed through the penis into the general circulation.

An intravenous injection of nicotine (10 mgm.) led to stimulation being without effect. The dilatation then was not caused by the diffusion of AC from the antidromic stimulation of sensory nerves.

Our experiments led us naturally to consider some other points in regard to the phenomenon of erection.

We recorded the increase in pressure in a corpus cavernosum in the following manner. A large-sized hypodermic needle with a bore of at least a millimetre was forced through the coat of the corpus on a long slant, so that its opening lay in cavernous tissue. This needle was connected to an outlet from a 500 cc. Erlenmeyer flask which was stoppered and filled with saline. Through another hole in the stopper the point of a burette containing mercury was inserted. The burette could be made to deliver a fine stream of mercury into the flask, thus forcing saline into the corpus. A side connection between needle and flask led to a spring manometer. On allowing the mercury to flow, the pressure did not rise, as the small amount of fluid injected readily escaped by the veins. The nerves were stimulated while the mercury was flowing. On stimulating the nerves, the pressure rose slowly and blood passed back into the system; after the stimulation was over, the pressure fell. The manometer was immediately connected to the carotid artery so that in the tracing, (fig. 2), the two pressures are comparable. The pressure fell, but never to normal, due to clotting or obstruction after the stimulation was over, and in spite of the mercury pressure being maintained. It is evident that the relaxation of the small helical arteries of the corpus led to a great increase in internal pressure. The venous outflow was incapable of dealing with

the inflow. This, according to Kiss (1921), is due to the veins being compressed within the almost rigid outer coat. There was no evidence that the mercury pressure contributed to the height of the rise, when the veins were so compressed, and this and other like observations led us to feel that the pressure within the corpus might rise to as close to the carotid arterial pressure as is represented in figure 2.

Many experimenters have claimed that the contraction of skeletal muscles might occlude the venous outflow. Kiss, on anatomical grounds, claims that this does not occur in man. In the dog, however, the ischio-urethralis muscles are inserted into a thick sheet of fascia lying dorsal and about the dorsal veins, or the single vein formed by their union. These muscles, if the pubis is removed, exert a very definite pull when their nerves are stimulated, and might seem to be ideally disposed to compress the vein, as is stated to occur by Ellenberger and Baum. This point was subjected to experiment in the following manner:—The penis was freed, reflected caudally, the retractor penis was cut away and the ischio-cavernosus muscle

Fig. 2

on each side was carefully dissected free from the ischium, and the ischio-urethralis muscles cleared and a platimum electrode laid on each. Hence, on closing the primary inductorium circuit, both muscles were stimulated directly. The penis was then enclosed in a long plethysmograph coming down to the ischio-cavernosa muscles. Whether the penis was dilated by stimulation of its nerves or not, stimulation of the ischio-urethralis muscles did not lead to any change in the volume. Further, it could be readily seen, especially during dilatation, that contraction of these muscles did not occlude or apparently diminish the flow through the veins. Further, when it is considered that the fascia into which these muscles are inserted is also strongly attached to the underside of the pubis, it becomes evident that the muscles could not act as effective compressors of the veins.

During these experiments we made, however, an interesting observation, namely, that, particularly during stimulation of the dilator nerves, sudden sharp increases in volume of the penis occurred. After each spike-like rise, the volume did not fall to the level to be expected before the rise. It was observed that these rises were due to sudden, short spontaneous contractions

of the ischio-cavernosus muscles, which, owing to their somewhat spiral arrangement and in spite of their origin being cut away, could produce some pressure on the parts of the corpora lying beneath them, and thus force some blood into the plethysmograph. This action of these muscles may explain in part why it was believed that skeletal muscles could participate in erection, since both of us have observed that when efforts are being made to produce an erection, short, sharp and apparently voluntary muscular contractions occurred, after each of which a gain in the amount of erection was noticed. The other muscle which might aid in a similar fashion is the accelerator urinae, by compressing the proximal end of the corpus spongiosum, but owing to the freedom of its venous outflow this does not seem probable. The transversus perinei muscles in man, weaker than the ischio-urethralis of the dog, cannot be supposed to produce any such effect.

Stimulation of the dilator fibres unilaterally increased the flow from both dorsal veins almost equally, owing to the venous and possibly arterial anastomoses in the corpus spongiosum and glans penis.

Testut figures definite intercommunications between the adjacent faces of the corpora cavernosa. Kiss denies, on the basis of his post-mortem injections, that such exist. In studying the vasular supply, we made injections of methylene blue into one visceral branch only, after tying the other visceral branch and the other arteries as described above, either after washing out with a saline injection or after perfusion. Using any normal pressure we found methylene blue only in one corpus cavernosum, but by using an abnormally high pressure, 200 mm. Hg, we found a little blue in the other. In this case, however, the arterial twigs entering the ischio-cavernosus muscle of the other side were filled with blue. This can only have reached them though arterial anastomoses with the hemorrhoidal system. Such anastomoses readily account for the blue appearing in the interior of the opposite corpus. This experiment would tend to support the position taken by Kiss.

SUMMARY

1. Evidence is presented that the vasodilatation on stimulating the dilator nerves to the penis is due to a local hormonal mechanism. The evidence is not such as to show whether this is due to an acetylcholine-like mechanism or not.

2. Evidence is presented to show that erection is not due to a compression of the efferent veins by skeletal muscle action.

3. A suggestion is made that skeletal, ischio-cavernosus, muscular contractions may play some minor part in erection.

4. Evidence is presented that there is a rapid rise of pressure during erection within the corpora cavernosa, which may well make the venous outflow inefficient.

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STUDIES IN THE NUTRITIONAL ANEMIA OF THE RAT

IX. Observations on the Anemia of Pregnancy[1]

HOWARD H. BEARD AND VICTOR C. MYERS

From the Department of Biochemistry, School of Medicine, Western Reserve University, Cleveland

Received for publication July 27, 1933

As the result of difficulties temporarily encountered in securing satisfactory reproduction in our rat colony we were led to study the hemoglobin and red cell concentration during pregnancy. It was found almost without exception that an anemic condition was present, the drop in erythrocytes being much greater than that of the hemoglobin.

Experimental. Eighty-seven female rats were studied during the course of 138 pregnancies. An examination of the blood of each rat was made before mating and at weekly intervals thereafter until surviving young were weaned. Usually cell counts and hemoglobin estimations were made on each rat for three weeks after parturition. An anemic condition was present only during pregnancy and lactation. The technique of making the erythrocyte counts and hemoglobin estimations has been described elsewhere (1).

The animals were divided into four groups, as noted in table 1. The stock diet was essentially the diet B of Sherman and Campbell (2), ($\frac{1}{3}$ whole milk powder, $\frac{2}{3}$ whole wheat powder, with 1 per cent of the weight of the wheat flour, each, as NaCl and $CaCO_3$) plus lettuce three times per week. In addition to a daily consumption of 10 grams of these diets, groups 3 and 4 received 0.5 gram of liver extract or yeast.

Results. The average results for 52 pregnancies obtained in this study are given at the bottom of table 1. These are representative of the 138 pregnancies studied. Practically all of the animals showed a progressive drop in cells and in many cases in hemoglobin from the beginning of pregnancy until the birth of the young. At this time there were usually 4 to 5 m. red cells per cu. mm., and 10 to 11 grams hemoglobin per 100 cc., although in some cases the hemoglobin was practically normal. The red cells and hemoglobin returned to normal often as early as one week after parturition. Liver extract and yeast did not prevent the occurrence of

[1] A preliminary report of these observations was presented before Section N, American Association for the Advancement of Science, New Orleans, La., December 29, 1931.

TABLE 1

Showing the occurrence of anemia in pregnant rats

GROUP	RAT NUMBER	DATE	NON-PREGNANT		DATE	PREGNANT		DATE	NON-PREGNANT		DATE	PREGNANT		DATE	NON-PREGNANT		DIET
		1930–31	Hb. gm. 100 cc.	R.B.C. m.cu. mm.	1931	Hb. gm. 100 cc.	R.B.C. m.cu. mm.	1930–31	Hb. gm. 100 cc.	R.B.C. m.cu. mm.	1931	Hb. gm. 100 cc.	R.B.C. m.cu. mm.	1931	Hb. gm. 100 cc.	R.B.C. m.cu. mm.	
I	1	11/ 4	16 1	8 3	11/30	10 0	4 0	12/16	16 6	8 1	1/ 6	9 6	5 1	1/29	15.7	8 4	Stock
	2	11/ 1	16 2	7 8	12/ 1	10 0	4 2	12/16	14 3	7 6	1/22	11 1	4 1	2/10	16 3	8 1	Stock
	3	11/ 6	15 8	7 7	12/ 3	12 5	5 7	12/16	15 8	8 4	1/16	10 3	4 8	2/ 6	16 5	8 6	Stock
	4	11/16	16 1	8 2	12/ 6	16 6	7 9	1/18	15 8	8 2	1/29	14 9	8.1				Stock
	19	12/ 1	13 7	8 6	12/24	10 3	3 8	12/29	13 3	7 7	2/ 2	10 2	4 6	12/16	16 1	8 5	Stock
	14	12/ 2	16 6	8 1	12/19	9 1	5 1	12/29	13 3	6 8	1/ 7	10 5	7 5	1/22	15 5	8 6	Stock
	16	12/ 1	15 1	8 5	12/19	14 3	5 2	12/29	14 0	5 6	1/ 7	10 0	7 0	7/18	15 8	8 3	Stock
	20	12/ 6	16 2	7 7	12/20	14 3	3 7	12/29	15 4	6 1	1/ 7	12 5	3 8	1/24	16 2	8 1	Stock
	23	12/20	15 4	4 8	12/27	15 4	4 8	1. 7	16 1	9 5	2/16	15 4	4 6	2/27	15 2	8 8	Stock
Averages			15 7	7 7		12 5	4 9		15 0	7 6		11 6	5 5		15 9	8 4	
II	31	1/16	14 3	8 3	3/13	12 5	3 9	4/ 4	15 8	8 2	4/17	10 5	5 5	5/ 6	14 3	6 2	Stock + 0.5 mgm.
	32	1/16	13 2	8 2	2/20	11 1	4 2	3/13	14 4	7 1	4/11	12 5	4 6	5/ 6	12 5	7 8	Stock Fe* daily
	38	1/16	12 5	7 9	1/22	10 2	4 6	2/ 6	11 6	6 9	3/13	11 1	4 4	3/30	13 1	7 2	Stock
	36	12/20	14 1	8 2	1/ 7	8 3	4 9	1/16	12 5	7 3	3/10	11 7	4 2	3/27	12 1	6 9	Stock
	34	1/16	13 3	7 5	2/18	11 7	4 6	3/13	11 1	7 5	4/26	12 1	4 5	5/15	11 9	7 5	Stock
	39	1/16	12 5	7 3	2/ 6	11 2	4 1	2/22	12 3	6 9	3/18	13 6	4 3	4/ 3	12 7	7 8	Stock
Averages			13 3	7 9		10 8	4 4		13.0	7 3		11 9	4 9		12 7	7 2	
III	46	2/25	13 7	8 3	3/11	11 2	5 0	4/ 2	15 4	7 1	6/ 1	9 1	4 6	6/20	13 7	8 6	Stock + 0.5 gm.
	49	2/25	14 1	7 6	3/18	4 9	2 3	4/ 2	12 5	7 6	5/17	11 1	4 4	5/28	14 3	8 1	Stock liver extract**
	63	4/20	13 6	8 1	5/11	10 0	4 8	5/16	13 3	7 5	6/21	10 4	4 2	6/30	14 1	7 9	Stock daily
	48	2/26	14 7	8 8	3/17	11 1	4 6	3/25	14 3	7 4	4/16	11 8	4 1	4/23	13 6	8 3	Stock
	47	2/26	13 8	8 7	3/13	7 7	4 4	3/25	20 0	8 3	7/16	11 8	5 6	7/26	14 6	8 9	Stock
	62	4/27	14 6	9 1	5/18	5 5	2 6	5/28	11.1	8 2	6/28	14 4	6 9	7/11	13 7	8 8	Stock
Averages			14 1	8 4		8 4	4 0		14 4	7 7		11 4	5 0		14 0	8 4	

IV	50	3/ 1	16 1	8 3	3/23	8 0	2.8	4/14	15 1	7 6	5/18	9 1	6 5	5/30	14 8	7 9	Stock + 0.5 gm.
	55	4/11	16 8	7 9	5/ 6	8 1	4 9	5/13	6 3	8 2	6/26	8 7	3 9	7/16	16 7	8 9	Stock yeast daily
	58	4/19	17 1	8 7	5/18	10 0	7 7	5/28	11 8	8 1	6/27	11 1	4 2	7/14	16 2	8 3	Stock
	59	4/19	16 6	8 4	5/ 5	14 1	7 3	5/20	14 6	8 6	6/14	8 8	4 2	7/ 7	16 1	8 2	Stock
	60	4/19	15 9	7 8	5/ 1	10 0	4 8	5/22	16 2	7 9	6/21	10 0	5 9	7/ 7	14 7	7 8	Stock
Averages			16 5	8 2		10 0	5 5		12 8	8 1		9 4	4 9		15 7	8 2	
Averages for whole series			14 9	8 0		10 7	4 7		13 9	7 6		11 2	5 0		14 6	8 1	

* In a few experiments Cu was also employed as a supplement to the Fe.

** We wish to thank the Eli Lilly Company for the supply of Liver Extract no. 343 used in this study.

the anemia, nor hasten recovery after birth of the young, but judging from the average data for hemoglobin presented in table 2, iron did have a definite effect in preventing the hemoglobin from falling to as great an extent as in the other groups.

Discussion. The results obtained in this study are comparable to those of a similar investigation reported (after the present study had been completed) by Mitchell and Miller (3), who collected data on 145 pregnancies in 51 female rats, 32 of which were under observation before the first mating, while 11 animals were observed through the fourth and fifth pregnancy. In their series there was an average fall of hemoglobin from about 16.5 to 13 grams per 100 cc. during gestation, while during lactation the hemoglobin rose to about 17 grams per 100 cc. Neither Fe, Cu or Mn addition nor yeast were able to prevent the onset of anemia. They regard the anemia of their animals as a physiological condition, dependent upon pregnancy *per se*.

TABLE 2

Influence of certain dietary supplements on the hemoglobin of the pregnant rat

Average values

GROUP	HEMOGLOBIN		
	Non-pregnant	Pregnant	Drop during pregnancy
	grams	*grams*	*grams*
I. Control	15.6	12.1	3.5
II. Receiving iron	13.0	11.4	1.6
III. Receiving liver extract	14.2	9.9	4.3
IV. Receiving yeast	15.0	9.8	5.2
Average all groups	14.5	11.0	3.5

It would appear that pregnancy puts a heavy metabolic demand upon the mother rat. This is shown by the progressive drop in cells and hemoglobin as pregnancy advances, and the quick return to normal after birth of the young, a condition which may be found in the same rat through several pregnancies. It would appear further that this anemia may be somewhat analogous to the nutritional anemia occurring during early growth.

No blood volume studies were made in our series, but it is not believed that the changes observed can be wholly accounted for on the basis of hydremia. Although the small drop in hemoglobin observed in many of the animals might be due to an increase in plasma volume, the drop in hemoglobin was too great in fully a third of the pregnancies to be explained in this way.

The anemia of human pregnancy has recently attracted much interest.

Strauss and Castle (4) have given the subject careful study, and in a recent paper relate anemia (reduction in the total hemoglobin content of the mother's blood) to defective diet or gastric anacidity. They believe that the reduction in erythrocytes during pregnancy is probably due to hydremia and is not a true anemia. Strauss (5) in carrying this study to infants born to women suffering from hypochromic anemia has observed that, although these infants exhibit a normal blood picture at birth, they may develop a severe degree of anemia during the first year of life. He states that this form of anemia may be prevented by administering iron to the mothers during pregnancy or corrected by administering iron to the anemic infants. There would thus appear to be a direct relation between the nutritional anemia of infancy and anemia of human pregnancy in some cases.

The anemia which develops in the rat during pregnancy and early growth is quite analogous to the anemia which has recently been reported during human pregnancy and during infancy. The addition of iron to the diet of the rat during pregnancy appeared to retard the drop in hemoglobin as shown in table 2. The rat should prove a valuable experimental animal for further studies in this field.

SUMMARY AND CONCLUSION

Anemia was observed to develop during pregnancy in the rat. The reduction in hemoglobin averaged in excess of 20 per cent and exceeded 40 per cent in about one-third the pregnancies. The reduction in red cells averaged about 40 per cent. The addition of iron to the diet of the pregnant rat appeared to exert some retarding influence upon the fall in hemoglobin.

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A STUDY OF THE RELATION OF PANCREATIC DUCT PRESSURE TO THE RATE OF BLOOD FLOW THROUGH THE PANCREAS

A. LAWRENCE BENNETT AND EUGENE U. STILL

From the Department of Physiology of the University of Chicago

Received for publication July 20, 1933

Those who have concerned themselves with the mechanism involved in secretin and nerve activation of the pancreas have from the first considered the possible importance of relative changes in the rate of blood flow through the gland. It is well established that changes in the rate of blood flow through the pancreas do affect the rate of secretion. Anrep (1916) demonstrated the inhibitory effect on pancreatic secretion brought about by splanchnic stimulation or compression of the aorta. We have confirmed these findings in experiments in which we measured the blood flow and decreased it by means of hemorrhage. In one experiment a decrease in the blood flow (through the pancreas) of 57 per cent resulted in a 44 per cent reduction in the rate of pancreatic juice secretion. Likewise an increase in rate of blood flow was demonstrated to have a reverse effect, namely, to increase the rate of secretion.

Weaver, Luckhardt and Koch (1926) first prepared what was considered to be vaso-dilatin free secretin. When its action was demonstrated by Weaver (1928) the question of a possible histamine or histamine-like action of secretin seemed definitely settled in the negative. However, recently, Gayet and Guillaumie (1930) found that secretin activation of the pancreas was accompanied by an increase in the rate of blood flow. They reported an even closer correspondence between the secretory and blood flow rates when the gland was activated by nerve stimulation. More recently (1932) these investigators have performed several experiments in which they found that a preliminary decrease in the rate of blood flow accompanies secretin stimulation.

The relation of nerve activation of the pancreas to vaso-motor effects upon the gland has been difficult to determine. Francois-Frank, and Hallion (1897) observed an increased volume of the gland accompanying the secretion which was brought about by vagus stimulation and they ascribed this phenomenon to vaso-dilatation in the gland. On the basis of this finding they claimed to have demonstrated vaso-motor fibers to the pancreas in the vagi. Anrep (1915) showed that the vagi carry both

secretory and inhibitory fibers to the pancreas. After studying the effects of these two sets of fibers upon the gland he concluded that the increase in pancreatic volume resulting from vagus stimulation is due to a retention of juice either within the gland cells or within the duct system. It was his conclusion that there are neither vaso-constrictor nor vaso-dilator fibers to the pancreas in the vagi. By direct measurement of blood flow Gayet and Guillaumie (1930) have demonstrated a vaso-dilator effect in the pancreas as a result of vagus stimulation.

From time to time, we have made observations in our studies on the pancreas, which directed our interest to the relationship between blood flow and secretion. In a particular series of experiments in which we were measuring the blood flow through the pancreas (Still, Bennett and Scott, 1933), it was observed that a certain sample of secretin, would occasionally cause an increase in the rate of bood flow. On examining our data we found that if an increase in the blood flow occurred, certain other things seemed to be involved. 1. If the duct was twisted or the cannula in the pancreatic duct was obstructed by clotted pancreatic juice, the injection of secretin invariably caused an increase in the blood flow. In one case when the injection of secretin had failed to cause a flow of juice, we attempted to loosen the obstruction by injecting a small quantity of saline through the cannula. It was observed that immediately following the short period of increased duct pressure, the rate of blood flow increased markedly and continued to exceed the previous rate for a period of several minutes after the pressure was released.

By repeating this procedure on several dogs it was found that an increase in the pancreatic duct pressure caused by the injection of from 3 to 6 cc. of saline or pancreatic juice would consistently cause an increase of from 20 per cent to 233 per cent in the volume of blood flowing through the gland over an average period of 11.6 minutes after the pressure had been released. It was also observed that frequently this procedure was accompanied by a momentary pause in respiration and a slight dip or rise in carotid blood pressure. 2. If the latent period of the secretion was prolonged (probably the duct contained a loose clot or a very thick juice) the injection of secretin lead to an increased blood flow. 3. The first injection of secretin sometimes led to an increased blood flow while subsequent injections of the same secretion preparation were without effect on the blood flow. 4. When the latent period was short (about 1 minute) and the duct and cannula entirely open and free to discharge pancreatic juice, the injection of secretin caused no change in the rate of blood flow.

This led us to reëxamine the data from 50 experiments in which we had measured the rate of blood flow through the pancreas in a study of the metabolism of the gland, and to perform new experiments particularly designed to throw light upon this problem.

In these experiments dogs of 8 to 9.5 kgm. body weight were used. The anesthesia consisted of intramuscular and intravenous barbital-Na, amytal-Na or chloralose.

The half of the pancreas drained by the pancreatico-duodenol vein was completely isolated from the duodenum by a continuous row of ligatures between the two organs. The pylorus was ligated as was the duodenum below the pancreas. A kymographic record was made of respiration, carotid blood pressure, blood flow through the pancreatico-duodenal vein, and pancreatic secretion. The blood flow was measured by means of the automatic stromuhr described by Bennett and Still (1933). Heparin was used as the anticoagulant.

The most significant criterion of changes in blood flow in these experiments was considered to be the per cent increase in total volume of blood passing through the gland during the period of changed rate as compared with the volume which would have gone through the gland during the same period at the previous rate of flow. This is hereafter referred to as the per cent increase in blood-flow volume.

The results suggested the possibility of a reflex vaso-dilatation with the stretching of the duct wall as the necessary stimulus. In order to determine the possibility that normal secretory pressure is sufficient to equal or exceed the stimulus threshold an analysis of fifty experiments was made. Of these fifty experiments in which blood-flow records were made during secretin activation of the gland there were ten in which for some unknown reason the cannula failed to deliver the pancreatic juice freely. Either the latent period was greater than 2.5 minutes or no juice left the gland at all. In the remaining 40 experiments the latent period was less than 2.5 minutes and the quantity of juice secreted was sufficient to be considered entirely satisfactory. Assuming that the pancreatic duct pressure was higher in the 10 experiments showing unsatisfactory elimination of juice than in the 40 experiments with relatively free egress of juice it was found that in those experiments with high duct pressure the average per cent increase in the volume of blood flowing through the gland was 40 per cent as compared with 9.4 per cent in those with low duct pressure. Also the average period of changed blood-flow rate was 20.0 minutes in the former as compared with 13.3 minutes in the latter. The secretory pressure developed with the gland itself was the only pressure involved in these experiments.

In order to measure the duct pressure which the gland developed while effecting this vaso-dilatation phenomenon two experiments were carried out. The gland developed a pressure of 18 mm. Hg the first experiment and 18.5 mm. Hg the second. The average per cent increase in volume of blood passing through the gland at 18 mm. Hg pressure was 14.2 per cent as compared with 3.3 per cent when the juice was allowed to flow freely through the cannula.

DISCUSSION. As may be seen from the table of results, it is necessary to have an increased pancreatic duct pressure for only a few seconds in order to cause an increase of 20 per cent or more in the blood-flow volume over a period of several minutes. The fact together with the magnitude of the blood-flow change and also the observation of central effects on respiration and general blood pressure leads us to believe that there is a reflex mechanism involved. There remains of course the problem of determining the units of the reflex arc.

TABLE 1

A study of the relation of pancreatic duct pressure to blood-flow through the gland

DATE	EXPERIMENT	PROCEDURE	VOLUME INJECTED INTO DUCT	PANCREATIC DUCT PRESSURE	TIME PRESSURE MAINTAINED	BLOOD-FLOW CONTROL	BLOOD-FLOW AT TIME OF MAXIMUM CHANGE	CHANGE IN RATE OF BLOOD-FLOW	TIME FOR MAXIMUM BLOOD-FLOW CHANGE	TOTAL VOLUME CHANGE IN BLOOD-FLOW	PERIOD OF CHANGED BLOOD-FLOW	PER CENT VOLUME CHANGE IN BLOOD-FLOW
			cc.	*mm. Hg*	*min.*	*cc./min.*	*cc./min.*	*cc./min.*	*min.*	*cc.*	*min.*	*per cent*
10/ 8/31	1	Inject in duct			24/60	3.0	15.0	12.0	1	24.0	6	133
10/ 8/31	2	Inject in duct	6		20/60	2.7	12.4	9.7	1.3	25.2	7	233
10/ 8/31	3	Inject in duct	5		1	5.0	12.4	7.4	1	17.6	9	44
10/15/31	4	Inject in duct	5		51/61	5.0	11.0	6.0	1	32.0	32	20
4/28/32	5	Intra-v, Sec.		Pressure assumed to be high because of faulty elimination of pancreatic juice		4.10	6.03	1.93	4	94.7	43	54
4/30/32	6	Intra-v, Sec.				6.24	9.23	2.99	2	25.3	12	38
5/28/32	7	Intra-v, Sec.				7.62	10.97	3.35	7	46.0	30	20
6/11/32	8	Intra-v, Sec.				1.25	3.21	1.96	6	14.4	21	55
7/ 5/32	9	Intra-v, Sec.				10.45	25.40	14.95	6	99.5	18	59
7/20/32	10	Intra-v, Sec.				5.26	7.88	2.62	7	21.7	19	22
7/30/32	11	Intra-v. Sec.				8.85	37.80	28.95	3	130.2	16	105
8/ 2/32	12	Intra-v, Sec.				16.71	19.61	2.90	7	31.2	12	18
8/17/32	13	Intra-v, Sec.				15.15	21.90	6.75	5	34.0	8	29
8/20/32	14	Inject in duct	5		7/60	1.65	2.57	0.92	1.5	3.8	10	23
3/23/33	15	Inject in duct	3		24/60	7.45	27.95	20.5	0.5	34.8	6	78
5/11/33	16	Sec., duct open		0		2.68	2.68	Normal rate of change				0
		Sec., duct closed		18	10	2.06	2.77	0.71	6	14.8	37	19.4
	17	Sec., duct open		0		2.37	2.59	0.22	2	2.2	14	6.6
		Sec., duct closed		18.5	9	2.31	2.59	0.28	2	3.75	18.2	9.0

It may be that the vaso-dilator fibers which Gayet and Guillaumie claim to have demonstrated in the vagi are part at least of the motor side of this arc. Also it may be suggested that the increase in volume of the pancreas which Francois-Frank and Hallion first described is in reality a combination of the two effects, the vaso-dilatation being the direct result of distention of the gland by the accumulating juice. Likewise in the light of this

phenomenon it is easily understandable that carefully purified secretin preparations which are considered vasodilatin-free may occasionally cause quite a marked increase in blood flow when injected, simply because there is a momentary retention of juice for one reason or another. This would account for the variable results reported by Gayet and Guillaumie and also observed by the author. We, of this laboratory are thoroughly convinced that secretin may be prepared which is entirely vaso-dilatin-free. The two preparations B and 413 have shown an average increase in blood-flow volume of zero in twenty-five experiments. But in single experiments

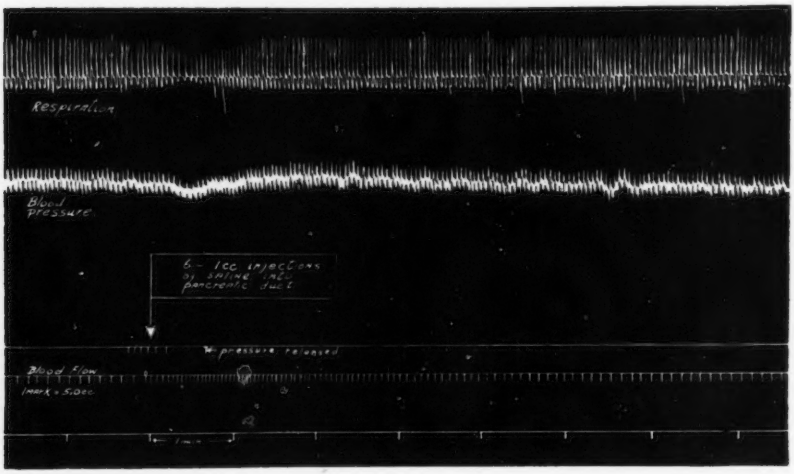

Fig. 1. A tracing showing the effect of raising the duct pressure in the pancreas by injecting saline upon the rate of blood flow through the organ.

sample B has produced as high as a 27 per cent increase in blood-flow volume.

CONCLUSIONS

1. Increased pancreatic duct pressure causes an increased rate of blood flow through the pancreas.

2. The pancreas, when secreting against an obstruction of the duct, is capable of producing pressure sufficient to cause an increase in the rate of blood flow through the glands.

3. The mechanism whereby increased pancreatic duct pressure causes an increased rate of blood flow through the pancreas is probably of the nature of a nervous reflex.

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FIBER GROUPS IN THE OPTIC NERVE

G. H. BISHOP

From the Department of Ophthalmology, Washington University, School of Medicine, St. Louis, Mo.

Received for publication July 31, 1933

While the anatomy of the optic tracts has been thoroughly studied, anatomical connections are obviously not sufficient to explain as complicated a process as vision. The anatomical pathway determines where an impulse arising from a sensory stimulus may go; it does not tell how it gets there, nor what alterations in the character of the impulse take place on the way, nor even under what conditions it will arrive at all. Recent advances in the technique of nerve physiology make it feasible to examine nervous impulses travelling over nervous elements in situ, while taking part in normal bodily activity. Where such responses are too complicated for analysis, simpler stimuli than those normally received from the surroundings cause simpler responses which may then be utilized in analysing more complex ones. These responses are recorded as electrical action currents, and it becomes important to know what type of action current each nervous element produces when playing its part in transmission of a complex sensory message to the higher centers. It is hoped to be able to describe visual sensation thus in terms of action currents; of the retina, of optic nerve fibers, of thalamus and cortex; and in terms of frequency of response, number and kind of elements involved, inhibition and facilitation of the pathway, etc.

The usual study of visual phenomena involves relating stimulus to sensation, or stimulus to response. But between stimulus and response many events occur as the sensory impulse traverses the nervous system, and it is often difficult to assign to a given nervous element, to retina, or to thalamus, for instance, its part in the complex whole, for our information as to what the different parts of the pathway contribute to the total effect has usually been indirectly inferred from anatomical structure or from deficiencies due to localized pathological lesions. The recording of action currents from these different regions of the pathway offers a possibility of studying the impulse *between* stimulus and final response, at stages where sensation and motor effects are not produced. This information to be sure will appear in terms of nerve action currents and not in terms of sensation, but relating these to cortical action currents will possibly enable us to relate them to the sensation to which cortical action currents correspond.

The present paper deals with the fibers of the optic nerve. Responses of the optic cortex to direct electrical stimulation of this nerve have been reported (Bartley and Bishop, 1933). The electrical responses of the retina to light and of the optic cortex to illumination of the retina are being investigated currently (Bartley and Frey). These unpublished results have been made use of here. For the present we are interested in the physiological characters of the different groups of fibers passing between retina and thalamus, as compared for instance, to the groups of fibers of other sensory nerves such as the saphenous.

Fiber groups in the optic nerve. It has been found in all nerves so far studied that the nerve fibers occur in groups, with relatively vacant spaces between them, and that these groups are related to function. For instance in the saphenous nerve, the group of fibers with fastest conduction mediates touch and pressure, a slower conducting group mediates pain and temperature and a still slower group is motor (Heinbecker, Bishop and O'Leary, 1933; Bishop, Heinbecker and O'Leary, in press). In the cervical sympathetic of the cat a fastest group is afferent containing vasodepressor fibers, a second slower group is motor to the nictitating membrane, etc., a third is motor to blood vessels, and for a fourth still slower no function has yet been detected (Bishop and Heinbecker, 1932). The vagus, cervical sympathetics, sciatic, etc., are similarly separated into groups, so generally, that the finding of such groupings in any nerve is circumstantial evidence at least that different functions are served by these groups. The optic nerve has three rather distinct groups of fibers, differing as in other nerves in conduction rate, threshold to electrical shocks, and other physiological properties.

Frog. The optic nerve of the bullfrog can be dissected out and studied in a thermostat, where the slowness of its deterioration indicates no serious interference with its functioning due to excision. (The rabbit optic nerve, in contrast to this, deteriorates upon cutting off its blood supply so promptly that it must be recorded in situ) (see below.) To record conduction rates and other properties precisely the longest possible distance is desirable between the stimulating cathode and the leading off electrode proximal to it. It is not necessary however that the anode of the stimulus or the distal lead electrode be on live nerve, and to obtain suitable distances between pairs of stimulating and leading off electrodes the nerve was dissected out with a strip of brain tissue on one end, where the anode was located, and the retina and a strip of choroid at the other, to receive the distal lead (fig. 1). The nerve was crushed in some cases at its entrance into the eyeball to render action potentials more or less monophasic. Since this procedure is quite ineffective for the slower fibers of the optic, as of other nerves, diphasic recording is perhaps quite as profitable. The distance of conduction between stimulating cathode and proximal lead,

including both areas under these electrodes, varied between 7 and 11 mm. in larger bullfrogs. Silver wire electrodes were employed. The amplified action potentials were recorded on the cathode ray oscillograph at a sensitivity of 200 mm. per m.v. or less, by photographing single records. The nerve was stimulated once per second.

Three main divisions appeared in the potential record of each nerve, corresponding perhaps to the A, B and C waves of other nerves, the B being identifiable as a B_1 or sensory B component by its short refractory period. The first two were both double but their two parts overlapped closely. The last was usually simple but in one case was distinctly double, and in another a small wave preceded the main elevation. The conduction rates of the fastest fibers of the first and second main waves were about as 1:3 to 1:4. Of these first four parts of two double waves, each was of approximately the same area, if anything the areas increased with decreasing conduction rates. This would indicate four groups of fibers of decreasing diameter, but of increasing number to the group, the number of fibers per group being presumably proportional to the inverse square of the average conduction rates of the potentials (Gasser and Erlanger, 1927). Since the conduction rates differed by a factor of two, if these fibers obey the fiber size rule of Gasser and Erlanger, there should be expected about four times as many fibers in any subdivision as in the preceding. Casual inspection of osmicated cross sections of the frog's optic nerve indicates an obvious preponderance of smaller fibers, but counts have not yet been made; several nerves have stained poorly in osmic acid, probably owing to poor penetration through the thick sheath and into so large a nerve.

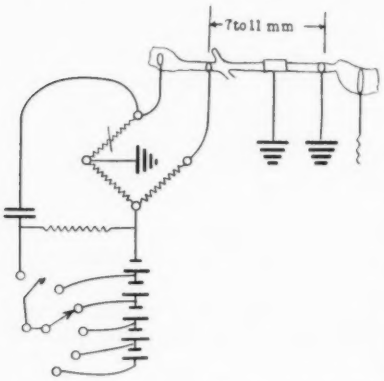

Fig. 1. Arrangement of excised frog optic nerve for stimulating and recording. See text.

There is no absolute interval in distribution of fibers, either by conduction rate or by threshold, the groups appearing by reason of alternate maxima and minima in a continuous distribution, until the range between the B and C is reached. Here there appears to be an actual interval in both threshold and rate, setting off the C group from the others. Since little is known about the area of potential per fiber in this range it is impossible to estimate the relative number of fibers in this last group; the total area of this wave is nearly equal to that of each of the two main potentials

preceding it, or nearly one-half that of all other potentials combined. The identity of the properties of these fibers with those of the corresponding fibers in other nerves, such as the saphenous, vagus and sympathetic, where the fibers can be inferred to be the non-myelinated ones of those nerves, suggests that here also the C wave of the frog's optic is assignable to non-myelinated axons.

On account of the short distance of conduction, these groupings were analyzed also by plotting area of potential against strength of stimulus. The nerve was stimulated by condenser charges; a 0.01M. F. condenser gave a threshold of 1.5 to 4.5 volts on different nerves, a smaller condenser usually being employed for the more irritable range. The time-potential areas were photographed by 1.5 volt steps of stimulus strength near threshold, by longer steps for the less irritable range, and by suitable voltage steps with a larger condenser for the still less irritable fibers, to obtain suitable increments of area per step. In interpreting threshold measurements it is necessary to recognize that changing condensers, which changes the duration of the stimulus, introduces an arbitrary factor. The ratio of voltages required to stimulate two given fibers through two condensers will vary with the capacity, the less irritable fiber being favored by the larger condenser so that the ratio of thresholds decreases with increased capacity.[1] This is merely to say that the apparent irritability to an electrical shock is least when the time constant of that shock matches the time constant of the fiber stimulated, and the less irritable the fiber, the longer the shock most suitable for its stimulation. With the short impulse that stimulates the most irritable fibers at say 3 volts, the least irritable in the optic nerve cannot be stimulated at 100 volts, while to use for the most irritable fibers a shock suitable for the least irritable may cause repetitive responses somewhat above threshold. In differentiating between fiber groups by means of threshold therefore the shortest convenient duration of stimulus is employed, and only the complete time-strength curves for each fiber or group of fibers could measure their relative irritabilities precisely.

In changing from a small stimulating condenser to a larger one, the last record from the former was repeated with the latter by choosing a voltage that gave the same amplitude of action potential measured on the oscillograph screen. The ratio of these voltages for equal effects through the two condensers was then used as a factor to convert the whole into one series.

[1] For instance, in one preparation the ratio of thresholds for the three main waves of the frog optic nerve was as follows: with a 0.005 mf condenser, 1:6.4, third wave not present; 0.01 condenser, 1:6:25; with a 0.25 sigma duration galvanic current, 1:1.8:8. In another preparation, for galvanic current of more than rheobasic duration, 1:2:4. The usual ratios for first and second potentials with a 0.01 mf condenser were about 1:4.

The ratio was not quite the ratio of the capacities, and the total quantity of current was thus not the same for the same effect with the two condensers, but was less with the larger condenser as should be expected with impulses of short duration.

Figure 2, B, is a typical plot of area against stimulus strength, on the same graph as that of amplitude of potential against stimulus strength (A).

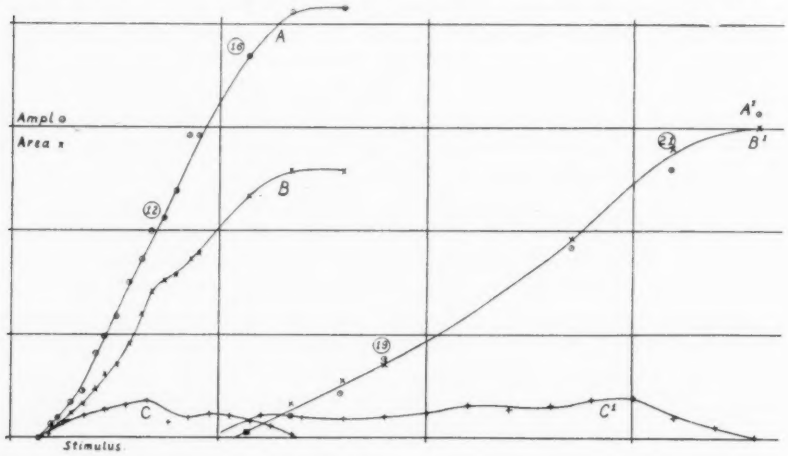

Fig. 2. Experiment 4/4/33. Curves A, A^1, first and second potentials respectively, frog optic nerve, conduction distance 9 mm., plot of amplitude on ordinates against stimulus strength as abscissae. Curves B, B^1, same potentials, plots of area of wave measured by planimeter against stimulus strength. There is a considerable error in the area curves due to partial diphasicity of the records, and it is not certain that this is a constant error, but the demonstration that the areas are more nearly equal than the amplitudes is presumably valid. Curves C, C^1, differentials of area curves, that is, plots of the increase of area per unit increase of stimulus strength, against stimulus strength. Note that between the first and second potentials, well separated in conduction rate even in a short nerve, there is a *continuous* increase of area with increase of stimulus. The significance of this lack of correspondence between rate and threshold is obscure at present. The same thing applies to the partial separation of the halves of each potential wave as they are observed on the oscillograph; a separation which is not obvious in the plots of total area or amplitude. A relative interval in conduction rate is more apparent than an interval of threshold or of area increase.

Figure 3 presents typical records from this series, the position of the records being indicated by numbers on the plotted curves. Conduction distance was 9 mm., rate of fastest fibers 16 m.p.s., of fastest fibers in the second main elevation 3.95 m.p.s. at 24°C. The stimulating condenser was doubled (0.005 to 0.01 mf) at record 15, before the appearance of the second potential. Since the graph plots integrals of areas with increasing stimulus

strength, the differential of this curve will plot the areas assignable to those fibers stimulated by each successive increment of voltage but not by the previous voltage (fig. 2, C). If threshold varies approximately as conduction rate over this range, the result should be similar in form to the photographed records of potential after conduction.

This is obviously not the case, and the discrepancy is being further investigated, since it shows up consistently in all series of records. The results indicate an interval in conduction rate without a corresponding interval in area of potential per unit of threshold increase.

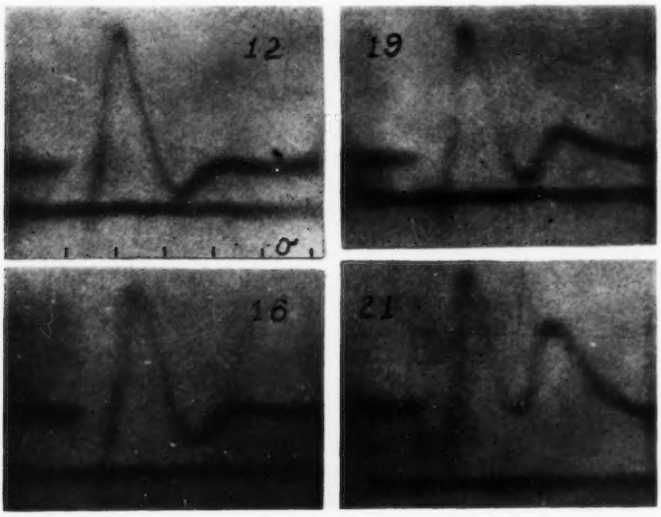

Fig. 3. Oscillograph records from series from which figure 2 was plotted, at points indicated by numerals in circles. Single deflections of oscillograph beam, time linear in sigma.

In figure 4 is presented diagrammatically the distribution of potentials in the optic and sciatic nerves of the frog.

Rabbit. In recording from the optic cortex of rabbits, stimulation of the optic nerve was found ineffective if the dissection of the nerve was too clean (Bartley and Bishop, 1933) and its failure was assigned to interference with blood supply. Since peripheral nerves of mammals survive excision with nearly normal activity, and since the optic nerve fibers appear to be quite similar in their physiological properties to fibers of peripheral nerves, in spite of the fact that the optic tract is ontologically a part of the central nervous system, and since the frog optic survived removal, this circumstance has been examined in more detail. The front surface of the eye was

removed, the rest of the eyeball dissected free from the recti muscles, and these fastened to the rim of the eye socket. The nerve was then dissected clean for 2 or 3 mm. from the eyeball, the latter was attached to the distal lead electrode, and the proximal electrode lay against the side of the eye socket. Since this was the ground electrode, the ground connection

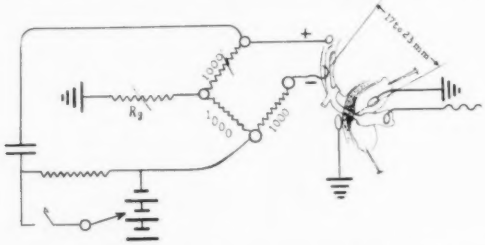

Fig. 4. Arrangement of electrodes for stimulating and recording optic nerve of rabbit in situ with blood supply intact. The resistance R_g in the ground connection of the bridge stimulator may be varied to obtain a lower stimulus distortion than can be obtained without capacity in the bridge arms otherwise. At one extreme (low) value of this resistance uncompensated electrode and tissue polarisation complicated the picture, and at the other (high) extreme, capacity in the stimulating circuit has a predominating influence, and the "balance" is a compromise between these.

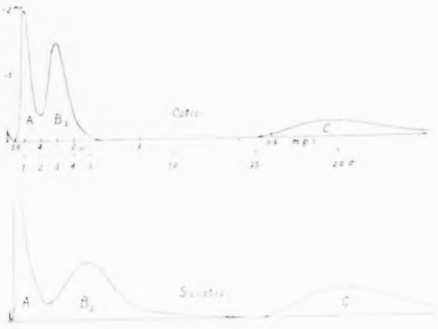

Fig. 5. Diagrammatic plot of conducted action potentials of frog optic nerve, approximately to scale for the various potentials, and for comparison (below) plot of potentials of the frog sciatic for same conduction distance. The A wave of the frog sciatic on this scale passes far beyond the limits of the plot.

with the nerve was effectively at the point where the cleanly dissected nerve left the mass of undissected tissue in the bottom of the socket (fig. 5). The action potentials of the first wave under these conditions, when the nerve was stimulated in the cranial cavity just behind the chiasma, were nearly completely monophasic. Similarly recorded from the frog, they were completely diphasic. The optic nerve of the rabbit had thus become

non-conducting between its emergence from the non-dissected tissue of the socket and its entrance into the eyeball. On reversing this procedure and stimulating just behind the eyeball, leading from the chiasma, the nerve was found non-irritable at the eyeball, but irritable one to two millimeters from its entrance into undissected tissue, the threshold decreasing toward the head. Presumably capillary circulation inside the nerve would extend out this far into the dissected region.

Rabbits were double-injected via the carotid with gelatin and starch masses, and the optic tracts were fixed, and cleared by the Spalteholtz method in wintergreen oil. There could then be seen the two main supplies to the optic tract. Vessels from the circle of Willis supply the chiasma, and the optic nerve out to its entrance into the eye socket. A branch of the ophthalmic artery enters the nerve within the socket, to form the central arteries passing to the retina, and capillaries from these anastomose with those from within the cranial cavity. (There is not a single central artery in the rabbit, but three or four small arteries which pass from the optic nerve to retina.) Several small vessels, both arteries and veins, lie rather close outside the surface of the nerve sheath, and pass along it to the eyeball to form the ciliary and other vessels. The arteries send branches into the nerve, and apparently the chief venous return from the nerve's capillaries is through these accompanying veins. It is obvious that clean dissection would strip these off, even if the main ophthalmic branch supplying the central arteries were left intact, and with isolation of the retina from blood supply other than that from the central arteries of the nerve, even with this arterial supply the blood cannot return to the venous system, and the nerve is rendered ischemic.

The question still remained whether loss of function as compared with other mammalian nerves could be due merely to the size of the nerve and the thickness of its sheath, preventing access of oxygen from the air. Within the cranial cavity the sheath is vanishingly thin, but even this stretch cannot be activated after removal from the body or injury to its blood supply in situ. Since at least the outside layer of fibers should be accessible to atmospheric oxygen, and since all the fibers fail, it must be concluded that the fibers of the optic nerve are in themselves more dependent on blood supply than are the fibers of other nerves in mammals. This is the only difference that appears, unless the thinness of myelin sheathes in the optic nerve be considered; while some fibers of other nerves have sheaths as thin, compared to fiber diameter, here all the fibers have relatively thin sheaths.

The foregoing procedure of dissection furnished as satisfactory a method as any for further recording, and was employed in most experiments (fig. 5). A second ground electrode was placed on the tissue of the head to reduce the stimulus artefact. The animal was decerebrated under ether

by removal of the cranial lobes through a large opening extending from the eye socket opposite the operated eye past the midline, the central vessels of the meninges being occluded by clamping the edge of the cranium with a heavy hemostat. The cerebral peduncles and anterior end of the brain stem were scooped out to allow the sides to fall in, bringing the optic tract between chiasma and superior colliculus upward and toward the midline, to facilitate placing a stimulating cathode on it. It could not be dissected free of the brain stem proper without destroying its blood supply; and to stimulate anterior to the chiasma left a very short conducting distance. The stimulating anode was placed anywhere nearby, off the optic tract, the cathode, a fine silver wire, was laid gently across the tract touching only its bulging surface and not in contact with the rest of the brain tissue. The third nerve to the operated eye was cut to avoid reflexly excited muscle action currents, which could intervene in time to disturb the record of a late wave. The stimulating electrodes were connected in one arm of a wheatstone bridge to reduce stimulus artefacts, which are more difficult to eliminate in a mass of tissue than in a linear stretch of nerve. After cessation of bleeding, which usually occurred in ½ hour, the electrodes and head were covered with dry clothes, and no artificial heat was applied in a room at 25°C.

The results of these experiments were less satisfactory than in the case of the excised frog nerve, in that conduction rates were more variable from animal to animal, and the preparations were liable to deteriorate for reasons unknown, but not difficult to surmise. However the records are very similar to those of the frog, allowing for differences of temperature, with the exception that the last wave (C) appears to be lacking, or extremely low. Two main elevations appear, each separable into two overlapping parts, as in the frog. Instead of the large C wave of the frog which at mammalian temperature should propagate at not over 2 m.p.s. (usually 1.5 or less in mammalian nerves under experimental conditions), a low diffuse elevation was discernible in two preparations whose start propagated at about 4 m.p.s. It occurred therefore in that part of the record where the frog optic nerve shows least activity, and would correspond in conduction rate to the fibers in other nerves which are autonomic myelinated ones. Whether this represents in the rabbit an element corresponding to the much more pronounced C wave of the frog cannot be said. (In the cervical sympathetic of the cat and rabbit, for instance, the same functions are served by postganglionic myelinated fibers in the one case, and non-myelinated in the other.) It probably can be stated, however, that in the rabbit optic nerve this late element is relatively poorly represented. The other alternative is that even in the best preparation, the operative procedure had resulted in the differential depression of the slow fibers, with little effect on faster fibers. In peripheral nerves the C potential is first

to disappear on deterioration of the preparation, and we have had difficulty in obtaining it for instance in human nerves, although it is present in fresh specimens.

Correspondence between groups of fibers in the optic and other nerves. Of the three main groups of fibers in the optic nerve of the frog, there can be no doubt that the first two are homologous with the first two in the rabbit. Allowance must be made for the possible interference even in the best experiments on the rabbit, with a factor,—blood supply,—which would affect the nerve's activity. The rates of the first potential vary from 50 M.P.S. to 20 at body temperature, and the normal rate in the body without operative interference might be above the higher figure. In both frog and rabbit, the ratio of rates for these two main waves is about 1:3.5, and this ratio is much less variable than are the measured rates. In the frog the rates for the first potential vary from 16 to 8 meters per second at 24°C. Taking the highest figures in each case as nearest normal, the ratio between frog and rabbit conduction rates is about 3. This is a reasonable figure for other known homologues in these animals at these temperatures, such as the first potential of the sciatic, that of the myelinated fibers of the sympathetic, etc. The third potentials cannot be dealt with similarly and their interpretation must wait upon investigation of their function in the body.

Comparing the optic nerve fibers to those of other sensory nerves such as the saphenous or sciatic, the first group of the optic nerve corresponds in conduction rate at least not to the first group in most sensory nerves, but to a slower element. In the rabbit, the rates are somewhat faster than those characteristic of the sensory B group of the saphenous, which mediates pain, temperature and vasomotor impulses; the second half of this first optic nerve potential approaches the B range. In the frog, the first potential is definitely not similar to the sciatic B potential (which is a motor sympathetic one), as indicated both by its faster conduction rate and by its shorter refractory period. It would correspond rather to the gamma wave of the A potential, and include what was once designated as the delta wave, no longer recognized by its original sponsors (Erlanger and Gasser, 1930) but considered by Bishop and Heinbecker (1930) to be homologous with the sensory B element of mammals. Here also the second half of the first potential of the frog optic nerve covers the range of a frog sensory element corresponding to the sensory B wave of mammals.

The second group, one-third or less the rate of the first, has no correspondence with any known prominent group in other nerves, of either frog or rabbit. The fibers of this group have conduction rates falling in the range of those of the motor B autonomic group in both animals, but in the optic nerve they are presumably not motor, and the refractory period is that of other sensory elements, and not more than one-third that of the

motor B element found elsewhere. The finding of a prominent group of fibers in a sensory nerve with the motor B range of conduction rates but with a sensory B refractory period[2] is of interest in further supporting the proposition that nerve fiber types can be identified by their physiological properties.

In this discussion of correspondence between properties of nerve fibers in the optic and other nerves there is of course no intention of suggesting functional homologies. It is intended rather to orient this work with respect to that on other nerves, and to indicate that in spite of the special character of the optic nerve as a brain tract, and in spite of its unusual dependence on blood supply, its fibers are the same sort of fibers that occur in peripheral nerves, and can be classified by the same criteria; the further general inference may then be drawn with more confidence that the grouping of fibers as indicated by distinct potential waves has a functional significance here analogous to the similar grouping elsewhere. This brings us to consideration of the third group in the frog optic, with no obvious homologue in the rabbit, but consisting of fibers that closely resemble known elements in other nerves, namely, the C fibers.

Since Cajal's work it has been recognized that fibers pass through the optic nerve in a centrifugal direction, but little is known of their function or character. Since metal impregnation stains are customarily used to study such tracts, and since not all the fibers are usually stained by these methods, it is not known how many nor of what histological aspect these fibers are. Cajal figures some rather thick fibers as motor, of which it can only be said that they appear thicker than non-myelinated fibers would appear in such preparations.

On the functional side, Arey (1916) studied in the fish Amieurus the movement of rods and cones upon illumination, and found that such movement took place in excised eyes and in situ, but not if the optic nerve alone were cut. Stimulation of the optic nerve however caused movement. He concluded that a double neural mechanism was involved, over the third nerve and the optic nerve; if the optic nerve were cut the normal activity of the third nerve prevented response to light, but the effect of the third nerve could be overcome by an effect of efferent fibers in the optic nerve. He stated, "It is certainly difficult to explain the rationale of a situation whereby an animal possesses a mechanism the components of which act antagonistically, thus allowing photomechanical influence to be exerted undisturbed" (p. 237).

While certainly difficult to explain, such a mechanism has obvious

[2] The refractory periods of the first and second potentials in one frog preparation were 0.81 and 0.94 sigma, in another, 0.88 and 1.17, in a third, two hours after dissection, 1.45 and 1.62 sigma. That of the third wave was 5 to 6 sigma, a reasonable figure for the similar wave in other nerves.

similarities to other antagonistic actions of the nervous system, in the gut, heart and circulation, where the balance or "tone" maintained by opposing nervous impulses can be modified at least by drugs, and apparently by circulating hormones, and possibly by other non-nervous agents such as food in the gut, and by the excitants of inflammatory processes in the circulation; quite as the movement of retinal elements in response to light in Amieurus takes place as a modification of the state of "tonus" produced by nervous impulses. This in fact seems to be a typical mode of action for the viscera; two antagonistic sets of nerve fibers maintain a degree of tone or readiness for further activity which puts the organ in a state easily and effectively responsive to a third external and non-nervous agent, the specific response to which, rather than to further nervous impulses, seems to be its characteristic function. The nerve fibers innervating such viscera are in general autonomic fibers. That is, they are such fibers as give action potentials falling in the motor B and C range, such as the third groups of the optic nerves of both frog and rabbit. The branch of the third nerve which supplies the fibers of the ciliary nerves in the rabbit, monkey and cat are all of this type histologically, small thinly myelinated and non-myelinated (unpublished work) and, of course, the third nerve innervation to the ciliary mechanism of the eye is parasympathetic. The objection may properly be raised that the retinal elements show no movement in mammals and many lower vertebrates; however, mechanical movement is not the only conceivable method of adjustment of the retina to illumination, nor is illumination the only conceivable condition with respect to which the retina might profitably be adjusted. The point is that as a visceral as well as a nervous structure, the retina is worthy of further study from the point of view of its possible innervation by autonomic nerve fibers. The secondary involvement of vision in various diseases and bodily states (for instance, gastric disturbances), suggests one method of approach.

As to the obviously afferent components, the presence of fibers in a nerve of special sense with properties quite typical of afferent nerves elsewhere, supports again Müller's doctrine of specific sense energies. In a previous paper (Bishop and Heinbecker, 1933) a preliminary account of the optic and saphenous nerve fiber distribution was accompanied by a consideration of a paper by Blair and Erlanger (1933) in which we disagreed with what we thought were their conclusions. This criticism proves to have been occasioned by our misinterpretation of their statement, and it is hereby withdrawn. Their further elucidation of the point criticised does not concern the present results, and we cannot see that it is irreconcilable with the doctrine of specific nerve energies. Our results indicate again as Müller inferred that the sensation experienced depends fundamentally on the nervous pathway involved. Nervous impulses do in fact differ in character, and the circumstance that groups of similar fibers mediate in a

given nerve similar functions indicates a further detail of the plan on which the nervous system is arranged. The fact that similar fibers in different nerves also mediate different functions indicates the uniqueness of the central connections, still allowing that where the fibers differ, adaptation of the various elements of a pathway to each other or to the functional requirements of the animal may be a valid purpose of those differences.

Relation of visual function to fiber grouping in the optic nerve. The findings as to fiber content of the optic nerve can be correlated at present only indirectly and remotely with eye function. The experiments of Bartley and Bishop (l.c.) in which the optic nerve was directly stimulated give us values for the threshold of stimulation which elicited detectable responses from the optic cortex, values moreover for stimulation of the nerve in situ, with approximately the same degree of dissection of the nerve stump as was necessary here. The lowest values certainly correspond in both cases to the most satisfactory blood supply in the nerve. The threshold for the first cortical potentials is of the order of the threshold for the *first* of the three nerve potentials, rather than for the second. However, the ratio of threshold for the first and second potential waves recorded from the nerve is greater than the ratio of threshold for the 2 potential complexes recorded from the cortex. The question is, can these potentials be considered to correspond, the first and second from the nerve to the first and second respectively from the cortex. There are two considerations that permit us to infer that they do so correspond.

First, while it is not feasible to record from the optic tract and from the optic cortex under precisely the same conditions of dissection and consequently of blood supply, there is good evidence that the threshold for first detectable cortical response is higher than the threshold for the most irritable fibers of the nerve. This evidence has been presented previously (Bartley, 1933); it is, briefly, that the threshold for the cortical response varies with the time at which the stimulus is applied during a rhythmic cycle of central excitability, and that this threshold can be considerably decreased by application of strychnine to the thalamus, without change in conditions of nerve stimulation. The indications are that it requires the summation of a considerable number of impulses to break through to the cortex, and since single shocks were employed as stimuli to the nerve, there is required, to obtain the requisite number of impulses for this summation, the stimulation of more than the lowest threshold fibers. The ratio of thresholds for the two cortical responses to nerve stimulation is therefore not required to be the same as the ratio for the two nerve responses that might be correlated with them, but might reasonably be lower.

Second, it is observed by Bartley (unpublished) that when the retina is stimulated by brief flashes of light, an action potential is recorded from the cortex that closely resembles the one which upon direct nerve stimula-

tion occurs at the higher threshold of the two. We can thus correlate a specifically visual sensation (which must be experienced by the rabbit following such a stimulus), a specific cortical potential, and possibly, the activity of a specific group of fibers in the optic nerve. The two quite different cortical potentials suggest two functional aspects of visual sensation, and the relative if not complete absence of one of these potentials in stimulation by brief light flashes presumably indicates that the fibers which upon direct nerve stimulation produce this, are not activated by such light stimuli. At least, these brief light stimuli activate via the retina relatively few of the larger fibers in the nerve, and relatively more of the smaller fibers. The situation suggests that one of two or more possible aspects of visual sensation is being emphasized, and the conditions of the experiment suggest that a sensation of brightness is what the rabbit would chiefly derive from the stimulus.

One is tempted to draw here an analogy with peripheral sensory nerves, although an actual correspondence is not justified by the facts at present available. In peripheral nerves a group of larger fibers mediates sensations of touch, including those permitting spatial discrimination, while a group of smaller fibers mediates pain and temperature (Heinbecker, Bishop and O'Leary, 1933). The latter sensations are those predominantly characterized by an intensity factor. By analogy one might anticipate that the larger fibers of the optic nerve would also mediate that aspect of vision concerned with spatial discrimination or form, while the smaller fibers would be concerned with the quantitative factor of intensity. The best that can be said of such a speculation is that there seems to be no serious objection to be made to it, and perhaps, that it suggests a point of attack for the further analysis of vision.

SUMMARY

The frog optic nerve contains three main groups of fibers differing in conduction rates and other physiological properties. The rabbit optic nerve contains two groups similar to the first two of the frog, and probably a third whose identification is less certain.

The first two groups of the frog optic nerve appear to be homologous with the first two of the rabbit nerve.

The fibers whose direct electrical stimulation in rabbits results in action potentials recorded from the optic cortex fall in the range of the first two groups; and it is probable that the activities of these two groups of nerve fibers are specifically related to the two different action potentials recorded from the cortex at different nerve thresholds.

The functional relationships involved in visual activity are the subject of tentative speculation.

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THE PRESENCE OF FIBRINOGEN AND PSEUDOGLOBULIN IN FIBRIN DIGESTS

WM. H. WELKER, GEO. GILMAN AND LUDVIG HEKTOEN

From the Department of Physiological Chemistry, University of Illinois College of Medicine, and the John McCormick Institute for Infectious Diseases, Chicago

Received for publication July 21, 1933

When fibrin is swelled in dilute HCl solution (0.02–0.03 per cent) and a small quantity of solid pepsin is stirred in, liquefaction proceeds so rapidly that the entire mass is liquefied in a few minutes (Welker, 1928). The thought arose that possibly this reaction might be a reversal of the change of fibrinogen into fibrin, but the tests of the filtrate of the digest with antifibrinogen serum gave no precipitin reaction.

It seemed of interest to study further the products of this rapid hydrolysis of fibrin. Beef fibrin was obtained from a slaughter house. It was difficult to wash this fibrin free from small clots, and all of the material had to be handpicked. After daily washing for a month the fibrin was swelled and digested rapidly with pepsin. The resulting fluid was filtered and precipitated at once at 50 per cent saturation with ammonium sulphate. The precipitate was dissolved in water and dialyzed free from the sulphate ion against distilled water in cellophane under toluol and dried at 40°C. Solutions of this dried protein proved to be antigenic, and the serum of rabbits injected with such solutions gave precipitin reactions in high dilutions of beef fibrinogen and beef pseudoglobulin, but not with beef serum albumin.

Experiments were now made with horse fibrin separated from oxalated plasma by adding calcium chloride. The fibrin was washed in cheese cloth on a washboard; imperfectly clotted material was picked out and discarded.

In two experiments the fibrin was suspended in distilled water with toluol on top and chloroform at the bottom, the water being changed daily for five to six weeks. The fibrin was then subjected to rapid digestion with pepsin after swelling in 0.02 to 0.03 per cent HCl. The resulting digests gave water soluble precipitates on 50 per cent saturation with ammonium sulphate. Solutions of the precipitates gave strong reactions with precipitin serum against horse pseudoglobulin, but only one gave a faint reaction with serum against horse fibrinogen.

Other samples of horse fibrin were treated in the same way but the wash-

ing in water was continued for more than three months when the fibrin seemed to be on the point of disintegration. The fibrin so washed swells less readily in 0.02 to 0.03 per cent HCl. The solutions of the precipitates on 50 per cent saturation with ammonium sulphate of the rapid digests in these cases reacted well with precipitin serum against horse fibrinogen as well as with serum against horse pseudoglobulin.

It should be stated here that the precipitates produced in fibrin digests on 50 per cent saturation with ammonium sulphate are not only soluble in water but also partly coagulable by heat. As would be expected from their content of pseudoglobulin and in some cases at least of fibrinogen, the solutions of these precipitates are antigenic and induce on injection in rabbits the liberal production of precipitin for pseudoglobulin and fibrinogen.

In the course of these experiments it was observed that when fibrin in the process of washing is left standing, free from bacterial contamination, it suddenly may undergo solution. This phenomenon suggests the presence in fibrin of a proteolytic enzyme which perhaps may be removed by washing with water. Fibrin loses the property of swelling in dilute HCl solution and of autolysis after a few minutes in boiling water. This phase of fibrin digestion will be studied further. The results of the following experiment with autolysis of beef fibrin are of interest because they show the liberation here also of fibrinogen and pseudoglobulin.

Crude beef fibrin was obtained from a packing house and washed free from blood. It was then placed in a settling jar with distilled water and protected by chloroform at the bottom and toluol at the top. The water was changed daily for two months and then every third day. Occasional biuret tests of the wash water gave negative results. At the end of four and one-half months the fibrin began to soften rapidly and pass into solution. Every few days for three weeks the material was filtered and the protein in the filtrate precipitated by 50 per cent saturation with ammonium sulphate. This precipitate was dried at 40°C. After drying the protein became comparatively insoluble in water but most of it dissolved in 10 per cent NaCl solution. Nearly all of the protein in such solutions came down on precipitation with 50 per cent ammonium sulphate. That the solution of this precipitate contained fibrinogen and pseudoglobulin is shown by its precipitin reactions: 1. The solution reacted well with the serum of a rabbit which had been immunized with a digest of horse fibrin; this reaction suggests the presence of fibrinogen because this protein is not species-specific (Hektoen and Welker, 1927). 2. The serum of rabbits injected with solutions of the protein from the autolysate of beef fibrin contained abundant precipitins for beef fibrinogen and beef pseudoglobulin.

What does the appearances of fibrinogen and pseudoglobulin in the fibrin digests mean? Is pseudoglobulin held as such in the fibrin by ad-

mixture or absorption so that it can not be washed away? And is fibrinogen, at least under certain circumstances, set free from fibrin as the digestion takes place? These and similar questions can not be answered now. Recent results indicate that fibrinogen may be associated very closely with pseudoglobulin and that pseudoglobulin may be concerned in the formation of fibrinogen and possibly also in its conversion into fibrin. If that should prove to be the case, the liberation of pseudoglobulin on the digestion of fibrin would seem to be explainable easily. It is of interest to note here that only an occasional trace of blood albumin has been met with in the course of many tests with potent serums against albumin of the protein fraction in fibrin digests, and there is no such association at all on the part of albumin with fibrinogen as in the case of pseudoglobulin.

The main outcome of these experiments is the demonstration that autolysis of beef fibrin and pepsin digestion of thoroughly washed beef and horse fibrins that have been swelled in 0.02 to 0.03 per cent HCl yield free substances with the antigenic properties of fibrinogen and pseudoglobulin. This result suggests that perhaps it would be of interest to study other digests also by means of the precipitin test.

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STUDIES ON THE PHYSIOLOGY OF SLEEP

X. The Effect of Alcohol and Caffein on Motility and Body Temperature during Sleep

F. J. MULLIN, N. KLEITMAN and N. R. COOPERMAN

From the Department of Physiology of the University of Chicago

Received for publication July 31, 1933

In 1883 Mönninghof and Piesbergen in studying what they termed the depth of sleep also made some observations on the effect of alcohol. They measured sleep by determining the intensity of a graded auditory stimulus necessary to awaken the subject. Following long walks and the ingestion of small doses of alcohol (four glasses of light beer), they found that the relative soundness of sleep was less than when no exertion or alcoholic enjoyment had taken place. It was otherwise on larger doses of alcohol without the walk, for sleep then was much sounder at the beginning and its total duration was longer. With these larger doses (no mention of the exact quantity is made) they found that sleep was much more restless than normal. Johnson and Swan (1930) confirmed these earlier findings stating that they "have direct evidence that a sleeper stirs considerably more frequently than normal if he is drunk when he goes to bed." They did not indicate what doses of alcohol made their subjects "drunk." In the same article they cite the work of Storm van Leeuwen, who reported that following the administration of an extract of sixteen grams of coffee his animals (dogs) showed a very marked increase in nocturnal activity, while decaffeinized coffee produced no such effect. Hollingworth (1912) studied the effects of caffein on sleep in adults, whose subjective reports were that with doses of from one to four grains there was no appreciable sleep disturbance, while with six grains there was marked sleep impairment. Karger (1925), in studying the effects of caffein on nocturnal motility in children, reported a very marked increase, but failed to indicate the amount of caffein administered. Stanley and Tescher (1931) found a slight decrease in nocturnal motility after one cup of coffee. Giddings (1933) noted that small doses of caffein had no appreciable effect on the motility of children during sleep.

We decided to reinvestigate the problem, using the methods described by Kleitman, Cooperman and Mullin (1933). This enabled us to measure the total time spent in movement, as well as the distribution of that time. We also recorded the total centimeter displacement of the bed-spring, and

the frequency and amplitude of individual movements. In some cases we took continuous rectal temperature records throughout the night and in one series a record of the number of times the subject awoke during the night. The room temperature was recorded before retiring and upon arising. All of the subjects used in these tests were perfectly familiar with the procedure and were accustomed to the sleeping room and recording apparatus.

PROCEDURE. In order to get the maximal effect of the alcohol used, 60 to 75 cc. of 95 per cent alcohol diluted to a 19 per cent concentration were taken forty-five minutes to an hour before retiring. On control nights for these series an equal volume of water was drunk at the corresponding time. The subjective element could not be ruled out, in that the persons tested always knew they were taking alcohol. They remained in ignorance of the results until the series was complete, however, so that a knowledge of past performance could not influence their sleep.

During the first part of the caffein work, coffee was used as a source of the caffein. The coffee drinks were brewed from varying amounts of ground coffee bean (25 to 75 grams) using three 5-ounce cups of water, percolated for seven minutes. One cup of coffee was taken at each of three consecutive forty-five minute intervals before going to bed. The series consisted of control days before and after twenty-five, thirty-six, fifty, and seventy-five grams of coffee. The subjects knew the strength (grams per cup) of this coffee, and this may have influenced their subsequent sleep. Because the caffein content could not be controlled when coffee was used (the caffein content of a given weight of coffee is not constant, even in the same brand, as stated in a communication from Hill Bros.), and in order to eliminate the subjective element, it was decided to use caffein solely. Doses varying from two to six grains were employed. The subjects usually were kept ignorant of the amount of the drug administered, being given three capsules of caffein, lactose, or a combination of both, each night, forty-five minutes before retiring.

Eight male subjects were employed in this study. The most extensive observations were made on ourselves, both as to number of controls and to number of drug records obtained. All of the subjects showed practically the same tendencies in their reactions to these drugs. Each subject was asked to write his own estimate of the night's sleep, directly upon arising and before he had seen the results as recorded by the various instruments used,—how he had spent the night, whether restlessly or quietly, and also the difficulty or ease with which he had fallen asleep.

Throughout this study the control nights and drug nights were irregularly intermixed in short series so that the records would be strictly comparable. This accounts for the difference (usually seasonal) in the controls for the two drugs, but it enables us to bring out more clearly the effects

produced on motility and body temperature during sleep by alcohol and caffein in the doses we used in this study.

Subjective observations. In each case the alcohol gave definite subjective effects and a feeling of intoxication, especially after the larger dose. All individuals reported that on going to bed they went to sleep practically immediately. This was especially marked in two of them who usually experienced some difficulty in falling asleep under ordinary conditions. The subjects were not habitual users of alcohol, and only two of them occasional indulgers. The subjective report of the person upon arising was usually of no change or else of a better night's sleep after alcohol, but some complained several times of a dull headache next morning.

Marked individual differences were noted in the reports made out by the subjects in the coffee and caffein experiment. N.K., an abstainer from coffee, complained of a "miserable night" after the consumption of three cups of the beverage made from a total of twenty-five grams of ground coffee bean. On the other hand, N.R.C., who occasionally used coffee, reported a normal night's rest after the same amount as taken by N.K., and even after taking quantities as high as thirty-six grams for three five ounce cups of coffee, but did complain of some degree of restlessness after taking fifty and seventy-five grams per three cups. F.J.M., not a coffee drinker, detected no subjective difference in his sleep after the smaller doses of coffee.

In the work on caffein N.R.C. was subjected to a preliminary series of experiments lasting thirty days. The subject recorded his estimate of the dose of caffein, such as zero, two, four and six grains, and the quality of his sleep. In his reports we find that in 47 per cent of the cases the estimate as to the number of grains of caffein taken corresponded to the actual number of grains given. His estimates were high 40 per cent of the cases, but in half of these approximations he made an error of only two grains. In 13 per cent of the trials the approximation of the dose was low, but here too in half of the cases he made an error of only two grains. For eight of the fourteen nights when no caffein was given an average of four and a half grains was subjectively estimated, two of the nights being very restless with a report of six grains each night. A correct estimate of zero grains was recorded for the remaining six of the fourteen drugless nights. (These fourteen nights were not consecutive ones, of course.) His subjective observations reveal a tendency to attribute restlessness on drugless nights to the action of caffein. With higher doses of caffein all of the subjects reported greater difficulty in going to sleep, more restlessness during the night, and an inability to fall back to sleep readily on waking during the night. With the smaller doses of caffein the subjects usually reported a slight restlessness or else no change.

Objective results. The systematic study of coffee was made on only two

subjects. In the case of N.K., motility and the time spent in such motility were so markedly increased by the smaller doses, twenty-five and thirty-six grains of coffee per three cups, that in order to avoid unnecessary discomfort the larger doses were not tried. With N.R.C., however, no such marked effects were noted on the smaller doses (table 1). Increasing the strength of the coffee caused a regular progressive increase in the time spent in motility after the drug as compared to control nights. Taking all of the coffee nights together there was a marked increase over the normal as to the number of movements executed by the sleeper.

TABLE 1

The effect of varying amounts of coffee on motility during sleep

		TIME SPENT IN MOVEMENT PER HOUR	DIFFERENCE BETWEEN A AND B	NUMBER OF DAYS	TOTAL NUMBER OF MOVEMENTS PER NIGHT
		seconds	*per cent*		
Series I a	Control	32.4		12	
Series I b	25 grams coffee	31.4	−3.2	4	
Series II a	Control	32.2		12	
Series II b	36 grams coffee	35.8	+11.2	3	
Series III a	Control	27.2		5	
Series III b	50 grams coffee	34.1	+25.3	2	
Series IV a	Control	36.0		10	
Series IV b	75 grams coffee	51.2	+42.2	3	
Total	Control			39	38.07
Total	Coffee 25-75 grams		+34.38	12	51.16

(Subject N.R.C.) Series I included 12 control days and 4 coffee days, so distributed that 2 control days preceded and one followed each of the coffee days. In series II, each of the 3 coffee days was both preceded and followed by 2 control days. Series III and IV were arranged in a similar manner. "Total control" denotes the average total number of movements per control night (7 hours), and "Total coffee" denotes the average total number of movements per coffee night (also 7 hours).

After alcohol the total time of motility showed no significant change except in the case of F.J.M., where there was a decrease of 16 per cent on alcohol nights. Caffein in the four and six grain doses gave an increase in the time spent in moving about (table 2). In the two grain doses caffein usually produced a decrease in the time spent in motility, in one case, N.K., the reduction amounting to 14 per cent.

The distribution of the time given to stirring showed, however, that although alcohol did not affect the total, it significantly decreased the time in the first half of the night and increased it during the second half. In

one control series with F.J.M. as the subject, sixty-seven per cent of the total time spent in movement occurred during the last half of the night, while on ten alcohol nights this was increased to 74 per cent. With caffein, on the other hand, the larger doses caused a considerable increase of the time spent in motility during both parts of the night, the greater increase

TABLE 2

The effect of alcohol and caffein on motility and temperature during sleep

SUBJECT	CONDITION	NUMBER OF NIGHTS	NUMBER OF SECONDS OF MOVEMENT PER HOUR	NUMBER OF CM. BEDSPRING DISPLACEMENT PER HOUR	TEMPERATURE LEVEL		NUMBER OF MOVEMENTS	
					1st half of night	2nd half of night	1st half of night	2nd half of night
B.O.B.	Control	6	9.5	5.9	—	—	13.1	26.2
	Alcohol	5	10.1	5.2	—	—	10.1	28.3
N.R.C.	Control	20	40.1	12.1	98.35	97.91	21.6	28.7
	Alcohol	13	42.3	11.7	97.63	97.93	14.7	31.2
N.K.	Control	15	42.3	15.2	98.46	98.48	14.6	23.8
	Alcohol	15	41.8	14.6	98.37	98.91	12.1	25.6
F.J.M.	Control	30	19.6	7.5	98.70	98.34	13.7	25.5
	Alcohol	30	16.9	6.8	98.52	98.55	11.5	26.3
N.B.	Control	8	31.5	11.1	98.53	98.27	12.3	22.8
	2 grains caffein	6	27.0	9.9	98.58	98.28	9.9	17.2
N.R.C.	Control	20	44.7	9.9	98.12	98.07	24.2	34.0
	4 and 6 grains caffein	19	50.1	10.8	98.26	98.16	30.2	39.4
	2 grains caffein	8	43.1	9.0	98.22	98.10	22.1	32.8
N.K.	Control	6	50.5	15.8	98.44	98.29	15.1	22.5
	4 and 6 grains caffein	4	58.3	17.5	98.63	98.53	20.0	33.4
	2 grains caffein	5	43.6	13.4	98.59	98.32	13.3	19.4
F.J.M.	Control	20	20.7	7.0	98.44	98.12	14.3	24.8
	4 and 6 grains caffein	16	24.9	8.9	98.78	98.35	19.9	28.9
	2 grains caffein	8	21.1	7.1	98.49	98.15	14.9	24.7

coming in the first period, however. The two grain doses, decreasing motility, did not affect one half of the night any more than the other.

The frequency and amplitude of movements occurring after alcohol and caffein showed the same types of changes as brought out by the clock method; alcohol decreased both the major and minor movements occurring during the first part of the night, the effect being particularly striking in the

first two hours, and increased those in the last half of the night. In subject N.K. the total number of movements occurring during the first two hours of sleep after alcohol decreased to 71 per cent of the controls; in subject N.R.C. the similar decrease was to 58 per cent; and in B.O.B. was to 64

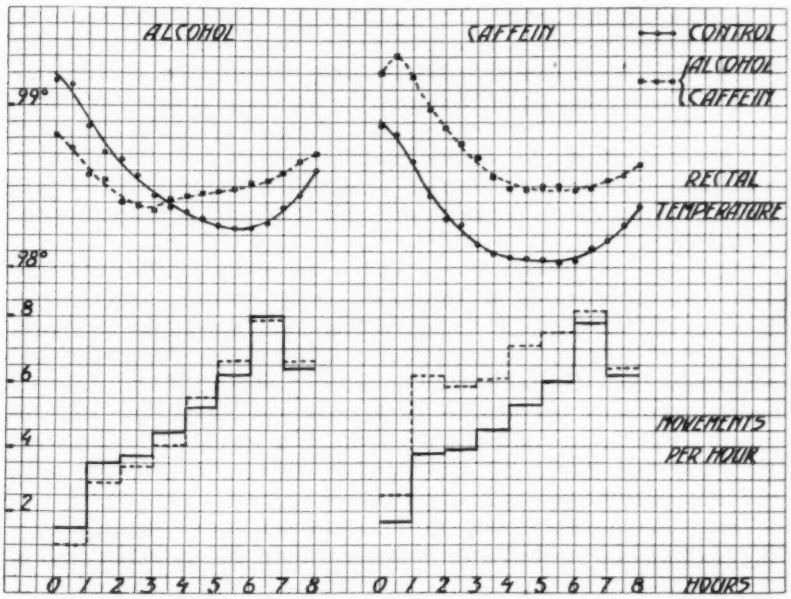

Fig. 1. The effect of alcohol (300 and 375 cc. doses of 19 per cent) and caffein (4 and 6 grains) on body temperature and motility of F. J. M. The temperature curves are based on the average values at the end of successive half-hour periods and the motility curves on the average number of movements during successive hours after going to bed. The alcohol records were obtained on a study of ten nights, with twelve controls; the caffein and its control curves are based on twelve nights each. Plotting all the individual points instead of the averages would give in the two cases distinct temperature bands, which also show these same variations and do not overlap. Only the larger doses were plotted for the caffein, because the two grain values did not vary appreciably from the control. It will be noted that the control curves for the alcohol and caffein are different. This is due to the fact that the first series of experiments was run in the autumn and the second in the winter (see Kleitman, Cooperman and Mullin, 1933, fig. 2).

per cent. In the case of caffein, the large doses gave a great increase in the number of movements occurring during the first half of the night, often because of the inability of the subject to go to sleep. In our experience, the greatest relative increase in motility seemed to occur in the second and

again during the fifth hours of sleep. Two grains of caffein usually caused a slight decrease in the number of movements occurring during both halves of the night. For the motility and temperature records after alcohol and the larger doses of caffein of F.J.M., see figure 1, which is typical of all the subjects.

The total displacement of the bed-spring in one direction, as measured by the Harvard work-adder and counter, shows a slight decrease after alcohol (table 2). This would seem to indicate, since the time spent in motility is about the same, that alcohol decreases the amplitude of the major movements. The larger caffein doses gave an increase in the total amount of movement as obtained by this method in all subjects. The two grains of caffein caused a slight decrease or else no significant change. In the case of N.K. two grains gave a displacement figure which was 84 per cent of the control figure.

The relative frequency with which one wakes up during the night after these drugs as compared to the control nights shows that alcohol again decreases and the four and six grain caffein doses increase the activity of the sleeper. All of the subjects reported quieter sleep after alcohol and restlessness after the larger doses of caffein. Subject F.J.M. woke up enough to press the recording key on an average of five times per night for twenty control nights, three and a half times for ten alcohol nights, seven and a half times for ten caffein nights of six or four grains, and five and a half times after the two grains of caffein (five nights).

The typical effects of these drugs on body temperature are shown in figure 1. Alcohol always caused a lowered temperature during the first half of the night and an increased temperature during the second half. This was true of all the subjects, no matter what their normal individual temperature curves were like. To study the effects on body temperature of similar doses of alcohol during the day time a series of ten controls and ten alcohol records was obtained using the same recording thermometer. The subject sat in a chair reading and writing throughout the day. After alcohol there was a distinct drop in body temperature as compared to the control, 1°F. in two to three hours, with a gradual return to normal in about five to six hours, but not above it. On two days, however, during a hot spell when the room temperature was nearly a hundred degrees no such marked drop followed the ingestion of the alcohol. The large doses of caffein always gave a significantly higher temperature at corresponding times during the night's sleep, while two grains gave only a slight variation from normal in all the subjects.

Discussion. In practically all cases the objective deviations from control nights did not correspond to the marked subjective effects of the alcohol before retiring and the feeling of having slept better on alcohol than on control nights. A difficulty in estimating the value of the objective results

lies in the fact that no definite criterion has been established as to what is an optimum amount of movement during sleep. The use of only the Telechron electric clock system in conjunction with the work-adder and counter failed to bring out an important point in connection with the distribution of motility and body temperature as affected by alcohol and caffein. But by the use of the fifteen-second marking signal magnet and the recording device we were able to see that following alcohol, though the total motility and the time spent in that motility was often not significantly different from control nights, the distribution of the motility was quite altered and a different temperature curve obtained. The period of relative immobility occurring during the first part of the night (as shown by the distribution of movements, the frequency of the fifteen-second signal magnet records, the incidence of spontaneous awakening, and the occurrence of uninterrupted periods of rest of thirty minutes or longer) was in all cases prolonged after alcohol, 75 cc. being more effective than 60.

The difficulty which one has in falling asleep after large doses of caffein is held by Stevenson, Christensen and Worts (1929) to be due to a decrease in intra-cranial pressure. This difficulty certainly depends to a considerable extent upon the previous activity of the subject, for in the case of F.J.M. it was often found that subjectively and objectively even the large doses of caffein had no marked effect on his sleep, yet on nights when he was worried about something or intensely preoccupied with work before taking the caffein, even the smaller doses were followed by difficulty in getting to sleep, restlessness, and frequent and prolonged awakenings during the night. Such sleep disturbances practically never occurred in this subject unless the previous nervous state was combined with caffein. The two grain doses showed usually no change or a slight decrease in movement during the night, which is in agreement with the results of Stanley and Tescher (1931), who found that one cup of coffee (5 grams per cup) taken before going to bed caused a decrease in nocturnal motility. A few records we have taken indicate that a cup of warm water before retiring also seems to lead to a slight decrease in movement during that night.

In our previous communication (1933) we gave evidence of a parallel seasonal variation in both the temperature level and motility. The connection between these two factors is brought out in an especially striking manner by the dissimilar effects of the two drugs studied here. Caffein raises the temperature level throughout the entire night and has the same effect upon motility. Alcohol lowers the temperature level during the first half of the night and raises it during the second part, and motility is affected by it in exactly the same manner. One can hardly escape the conclusion that there is a causal relationship over a period of time between these two factors, even though we have demonstrated conclusively that individual movements and body temperature changes are in no direct

way connected with each other and despite the fact that whereas the body temperature during sleep first goes down and then up the motility curve shows an upward trend throughout the entire night. Perhaps the cramping effect of repeatedly assuming the same or similar positions makes itself felt more and more as the night progresses, and is thus added on and serves to mask the parallelism which should exist between the temperature level and motility.

We are unable to account for the increase in body temperature the second half of the night following alcohol (fig. 1). It may be that as the effect of the drug wears off the loss of heat by peripheral dilatation is more than balanced by the increased production of heat due to the oxidation of the alcohol. The prolonged period of relative immobility during the first part of the alcohol night may be the cause of the increased activity and temperature during the second half.

We have some evidence that lying still for a considerable length of time leads to an unpleasant feeling which is relieved on moving about. On several occasions when he woke from sleep feeling cramped and fatigued, subject F.J.M. noted on examining his record that he had been lying in one position for a considerable length of time (an hour and a half or more usually) on every occasion when this occurred. One other subject, L.M., who slept in the laboratory for three successive nights in an attempt to demonstrate that he could sleep without stirring, succeeded in doing so, but gave up the experiment because he felt too "tired and groggy" after each night's sleep. This unpleasant feeling that comes from having slept in one position too long is usually relieved only after the subject stretches and moves about a bit. This may be the factor, or one of them, which causes the greater amount of movement during the last half of an alcohol night as compared to the control night. The fact that the alcohol curve obtained during the daytime hours, when the cramping effect of holding one position for a long time is absent, shows a return to normal but no increased temperature afterwards seems to point to the probability that the increased temperature during the last half of an alcohol night may be due to increased tonus and motility caused by the unusual lack of stirring in the first half of the night.

SUMMARY

1. Alcohol (300–375 cc. of 19 per cent) has either no marked effect or produces a decrease in the time spent in movement during sleep.

2. The alcohol causes a distinct reduction of motility and body temperature during the first half of the night, with an increase in both over the controls during the last part of the night's sleep.

3. Large doses of caffein (4–6 grains) produce a marked increase in motility and body temperature during sleep.

4. Small doses of caffein (2 grains) cause no significant change in the body temperature during sleep, and usually cause a slight decrease in the nocturnal movements of the subject.

5. Alcohol gives a subjective impression of having slept better, while the larger doses of caffein produce disturbed sleep.

We wish to thank Messrs B. O. Barnes, N. Brewer, A. I. Doktorsky, L. Morscher and S. Platt for their assistance in serving as subjects during this study.

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SIMULTANEOUS STUDY OF THE CONSTITUENTS OF THE SWEAT, URINE, AND BLOOD; ALSO GASTRIC ACIDITY AND OTHER MANIFESTATIONS RESULTING FROM SWEATING

XI. Phosphorus and Sulphur

G. A. TALBERT, F. STINCHFIELD and H. STAFF

Received for publication July 25, 1933

The report made by Langlois (1) and others many years ago that the sweat contained only about 0.01 mgm. in sulphates in 1000 cc. and only a trace of earthy phosphates and sodium phosphates caused us to re-examine the subject by more delicate and accurate modern methods.

We used the Fiske and Subbarow (2) colorimetric method for the determination of the amount of P_2O_5, and by the use of the factor we found the amount of phosphorus. For the estimation of the sulphates we used the Folin (3) $BaSO_4$ precipitation method and calculated the amount of sulphur correspondingly. The method of collecting the sweat was the same as that of our previous experiments (4).

We have made upwards of 300 determinations of phosphorus in the sweat from 18 different subjects and we have found appreciable but variable amounts of both inorganic and organic origin.

However, this report is selected from the last 100 analyses upon six different subjects. It is true in a very few cases we obtained negative results. While as a rule the total phosphorus yielded 1 to 2 mgm., yet in several instances an excess of 3 mgm. per 100 cc. of sweat was obtained. In the few cases where we had work sweat we found the phosphorus ran higher than heat sweat. In one instance 4.8 mgm. to 100 cc. was recorded. Likewise sulphur was found in quite as variable quantities. It was discovered to be from as low as 0.7 to as high as 7.37 mgm. per 100 cc. of sweat.

In the 94 analyses where it was possible to check the total phosphorus with the total sulphur from the same sample of sweat, in 70 per cent of the cases the latter was slightly in excess. In some instances where phosphorus was in excess, this excess was due to work sweat as stated above, while another portion of this excess probably finds an explanation in the discussion which follows. The more frequent approximation in their values was apparent when their concentrations were low.

The relation between these two elements or their respective relation to the excretion of calcium or magnesium is scarcely predictable. Whatever the disturbance there might be in the equilibrium of calcium and phos-

phorus in the blood by the intake of phosphates as contended by Binger (5) and others, does not seem to find its counterpart in the excretion of these elements in the sweat.

During the past nine years the major author of these experiments with his collaborators has made a most earnest endeavor to check the constituents that are common to the sweat, urine, and blood for the purpose of ascertaining whether there is any correlation of these constituents in these fluids. On the whole such expectations have been disappointing. Furthermore, while it is well known that in some instances where elements or compounds are taken by mouth or otherwise bear a certain amount of correlation to the excretion of these in the urine there is practically no such correlation with the sweat. That does not mean that the intake of a

TABLE 1

SUBJECT	1 LOW PHOSPHORUS	2 HIGH VEGETABLE PHOSPHORUS	3 EGG YOLKS	4 FISH	5 H_2NaPO_4
C.	1.84	2.08	2.92	3.23	2.7
C.	2.08	2.28	3.36	3.34	2.91
Fi.	1.04	1.84	2.40	3.03	3.09
Fi.	1.77	2.49	2.50	3.23	2.91
L.	1.55	2.85	2.90	3.12	3.34
L.	1.84	2.77		2.70	3.12
J.	1.46	2.50	2.90	2.92	2.88
J.	1.56	2.10	2.92	3.03	2.91
Fl.	2.12		3.29	2.29	3.08
Fl.	1.77	2.27	2.55	3.12	3.37
M.	2.18	2.27	2.53	2.91	3.32
M.	2.08	2.50		2.91	

high concentration of certain constituents in some instances does not find an increase in the sweat.

Our attention has been particularly drawn to this fact by the phosphorus excreted through the skin. We had been obtaining pretty regularly in our analyses a concentration of $\frac{1}{2}$ to $1\frac{1}{2}$ mgm. of phosphorus to 100 cc. of sweat, when on one occasion one of our subjects yielded about 200 per cent higher content than usual. Upon inquiry it was discovered that the subject had eaten a very large serving of fish the night before. This naturally gave us a suggestion of placing all of our subjects for two successive days on a high phosphorus diet and then alternating for two days on a low phosphorus diet. The results were of some significance as indicated by the table.

It did not seem desirable to be very precise in measurement of the high phosphate diet because of the variable amount that will pass out through

the alimentary tract. We did assure ourselves that there had been a very liberal serving of such a diet.

In the table the first column is to be considered as a control diet of a low phosphorus content. The second column contains a menu in which there was a liberal amount of vegetables of a high phosphorus content like spinach, asparagus, etc. The third column has for its high phosphate food one half dozen of hard boiled egg yolks. The fourth column was made up of a diet in which there was a very large serving of fish. The fifth column was the records received from the administration of 15 grams of Na_2HPO_4 a few hours before the sweating. The diets prescribed were taken the night before the experiments which were made early the next morning.

We have selected from six subjects two sets of experiments which are pretty well complete. These findings may be looked upon as fairly typical. It will be noted that the fish diet and the administration of Na_2HPO_4 give the highest reading while the egg yolk gives a lower one. The high phosphorus vegetable diet is lower still but yet is quite appreciably higher than the control diet. Possibly a reasonable explanation for the lower excretion of phosphorus through the skin in a diet made of spinach, etc., might be due to the increase in the fluidity of the stool, causing more phosphates to pass out through the alimentary canal.

We wish to acknowledge here that J. Walsh and E. Irgens made many of the determinations of the phosphates that are not included in this article.

SUMMARY

1. Phosphorus secreted through the sweat varies from a trace to 4.8 mgm. per 100 cc. and sulphur from a trace to 7.37 mgm. per 100 cc.

2. Phosphorus is increased in the sweat as a result of a high phosphorus diet and it is higher in work sweat than heat sweat.

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EYE REACTIONS IN PANCREATIC DIABETES

M. E. MacKAY SAWYER[1]

From the Laboratories of Physiology in the Harvard Medical School

Received for publication July 25, 1933

The effect of adrenalin, when instilled into the conjunctival sac, in producing dilatation of the pupil is strikingly increased under certain conditions. Loewi (1908) first observed that after complete removal of the pancreas in animals adrenalin produces a marked mydriasis, whereas before the operation it had no effect on the size of the pupil. Since that time clinical observations have been made to determine the diagnostic value of the test in cases of diabetes mellitus. It was found that only about 34 per cent of diabetics showed a positive response and that the correlation between the occurrence of the reaction and the severity of the disease was not apparent. A positive eye reaction was also sometimes observed in other disorders such as hyperthyroidism, gastro-intestinal disturbances, peritonitis, nervous lesions and nephritis (Lepehne and Schlossberg, 1924).

In the present investigation the increased sensitivity of eye structures (iris and nictitating membrane) to adrenalin has been studied in experimental diabetes with the object of determining whether this phenomenon indicates increased activity of the sympathetic system. Cannon, McIver and Bliss (1924) showed that over-activity of the sympathetic occurs in hypoglycemia. It seemed possible that in diabetes, although the blood sugar is high, a lessened ability of the cells to use glucose could produce a situation which, in respect to cells governing the sympathetic, would be equivalent to hypoglycemia. Thus the sympathico-adrenal system might be stimulated and the Loewi reaction would then become significant as an indicator of increased sympathetic activity. This question involved a consideration of not only the adrenals, but also the thyroid and liver as possible sources of thyroxin and sympathin respectively.

METHODS. Two test organs in the cat have been selected to indicate the secretion of adrenin, namely, the nictitating membrane (n.m.) and the iris. In experiments with the n.m., both superior cervical ganglia were removed about fourteen days before the animal was used. In these experiments, the first procedure was to learn the response of the n.m. on one side to different strengths of adrenalin instilled into the eye, and then to select a suitable

[1] Medical Fellow of the National Research Council.

dose for use throughout the experiment. The method of testing was the same as that described by Sawyer and Schlossberg (1933). Measurements were taken of the width of both membranes, when the animal's eyes were open and looking straight ahead, by holding a millimeter scale horizontally in front of and close to the eyes. One drop of adrenalin solution freshly prepared in distilled water was then instilled into one of the eyes and care was taken that the drop did not flow out. Readings of the width of the two membranes were made every two minutes until that of the instilled eye had returned to the same width as the control eye. To obtain comparative results with the same strength of solution on different days, it was important that the animal be not excited in any way and remain lying quiet throughout the test period. After a suitable dose of adrenalin had been selected and the reaction to it established on four or five different days, the pancreas was completely removed. The animals were usually kept a few days on insulin to permit recovery from the effects of the operation and then insulin was withheld. The reaction of the n.m. was determined each day after removal of the pancreas. A procedure similar to the above was used in experiments in which the iris was used as an indicator.

The conditions existing after pancreatectomy were quite constant in most of the animals. If insulin was administered daily in amounts sufficient to prevent the appearance of an abundance of acetone bodies in the urine, the animals survived for six or eight weeks. During this time there was considerable loss of weight, large amounts of fat were found in the urine, and the intra-abdominal and subcutaneous fat deposits were almost entirely depleted at the time of death. As a rule, however, animals were not kept long enough for considerable depletion of fat stores, for, if insulin was withheld, acetone bodies commonly appeared in the urine on the second day; and they increased greatly on the third and fourth days, when the animals often died. Usually animals of average nutritive condition were able to survive pancreatectomy not more than four days without insulin. Although those which died in acidosis did not go into coma, they showed quite characteristic symptoms before death,—dilated pupils, which in terminal stages reacted slightly to light, vomiting, increased heart rate, increased respiration and muscular weakness. The dose of insulin required to keep an animal from acidosis varied considerably (from 3 to 20 units daily) and depended on the amount of food taken and on the bodily condition (whether fat or thin). In most cases, while insulin was being administered an attempt was made not only to keep the urine free from acetone bodies but also to reduce the glycosuria to a minimum. In the majority of diabetic animals the daily total excretion of sugar was determined by Benedict's quantitative method and the acetone bodies by Rothera's test (qualitative).

RESULTS ON THE NICTITATING MEMBRANE. *The reaction in the normal*

animal. The amount and duration of the contraction of the sensitized n.m. produced by the instillation of adrenalin were found to be larger, the stronger the solutions employed (fig. 1). The dose of adrenalin which in most cases had a suitable effect (i.e., produced a contraction of the n.m. which could be definitely recorded and yet did not last more than half an hour) was in the range from 1:25,000 to 1:50,000. The criterion of the response to adrenalin was the length of time required for the n.m. to return to its original width. In addition to recording the width of the membrane, observations were made simultaneously on its minute blood vessels and on its color. In nearly every case the reappearance of the blood vessels and the attendant pale pink color of the membrane nearly coincided with the end of contraction and the return to the original pupillary width.

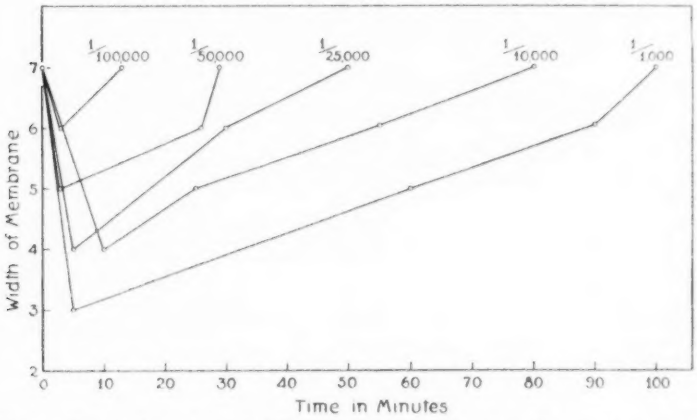

Fig. 1. Shows the effect of different strengths of adrenalin on the nictitating membrane.

Instillations of the same amount of a standard solution of adrenalin into the eye on different days evoked a relatively constant response of the n.m. in each individual. For example, in one cat the duration of the contraction produced by 1 drop of a 1:50,000 solution on five different days was 45, 48, 49, 50 and 50 minutes respectively. Similar results were obtained on four other animals.

The effect of removal of the pancreas on the response of the nictitating membrane to adrenalin. In the five animals mentioned above complete pancreatectomy was performed after the response of the sensitized n.m. had been determined. These animals were given insulin for a few days after the operation. During this time the effect of adrenalin on the n.m. in four of the animals was only slightly increased, while in the fifth it was slightly

decreased. On the first day after insulin was withheld very little change in the reaction occurred, but usually on the second day a definite increase in the duration of contraction was observed. This increase became quite striking on the third or fourth day without insulin, when the animals showed signs of acidosis and were excreting large amounts of acetone bodies in the urine. After the development of this extreme condition four of the animals were recovered by the injection of insulin; in three of these there was a decrease in the reaction of the n.m. as the acetone bodies disappeared, and in two of the three kept on insulin the membrane response finally returned to the normal measurements made before removal of the pancreas.

TABLE 1

Effect of adrenalin on the nictitating membrane before and after pancreatectomy

Cat S11. Strength of adrenalin (1 drop, 1:50,000)

DATE	DURATION OF MEMBRANE CONTRACTION	DOSE INSULIN	ACETONE IN URINE
	minutes	*units per day*	
November 2	24		
November 5	30		
November 10	29		
November 18	25		
November 18	Pancreas completely removed		
November 18–24		2–6	–
November 24	42	6	–
November 24–26		6–12	–
November 27		None	–
November 28	65	None	–
November 29	70	None	++
November 30	83	14*	+++++
December 1	123	16*	+++++
December 2	64	16*	–
December 3	34	12*	–
December 4	37	12	–

* After readings were taken on the membrane.

In table 1 the protocol of a typical experiment is shown. The duration of contraction of the left n.m., produced in four days by 1 drop of adrenalin (1:50,000), varied between 24 and 30 minutes. After pancreatectomy and the subsequent withdrawal of insulin the duration of contraction increased daily to 123 minutes. At that time the animal showed a marked acidosis. On the readministration of insulin and the disappearance of acetone bodies the duration of contraction was reduced to 34 minutes. In table 2 the summary of five experiments is given, showing the average duration of contraction of the nictitating membrane before pancreatectomy and the maximum duration of contraction observed after pancreatectomy.

A marked increase in the response of the n.m. to instilled adrenalin also occurred in two of these animals after an over-dose of insulin. In each case the insulin was administered shortly before the instillation of adrenalin, and a very pronounced and prolonged contraction of the membrane was observed 30 to 60 minutes before contraction of the control membrane, dilatation of the pupils or any of the other outward symptoms of hypoglycemia were observed.

The effect of inactivation of the adrenals on the response of the nictitating membrane. In one of the five animals described above (no. 531, table 2) the adrenal glands were inactivated (left denervated, right removed) and the liver denervated, after pancreatectomy. In this animal removal of the pancreas caused an increase in the duration of contraction of the membrane from 40 to 67 minutes. Readministration of insulin diminished the effect

TABLE 2

Comparison of the effect of adrenalin on the nictitating membrane before and after pancreatectomy

CAT NUMBER	DURATION OF CONTRACTION OF MEMBRANE		TIME OFF INSULIN WHEN MAXIMUM REACTION WAS OBSERVED	ACETONE IN URINE AT TIME OF MAXIMUM REACTION
	Average before pancreatectomy	Maximum after pancreatectomy		
	minutes	*minutes*	*days*	
527	40	95	3	+++
545	30	94	2	++++
S2	53	112	4	++
S11	26	123	3	++++
531	40	67*	5	+++
		105**	3	++++

* Before adrenal inactivation.

** After adrenal and liver inactivation.

to 40 minutes. During this time the adrenals were inactivated and insulin was again withheld. In four days the duration of the effect of adrenalin on the membrane had increased to 105 minutes, and the animal showed pronounced acidosis.

RESULTS ON THE IRIS. *The response of the normal iris to instilled adrenalin.* Because of the difficulty in standardizing the response of the n.m., the iris was used in the majority of experiments as an indicator of the sensitivity to adrenalin before and after removal of the pancreas. After some experiments on the responses of the iris, sensitized by previous extirpation of the superior cervical ganglion and made clearly visible by removal of the n.m., it was found satisfactory and more convenient to use the non-sensitized normal eye.

Although several investigators had reported that instillation of adrenalin

into the conjunctival sac of normal cats had no effect on the dilatation of the pupil (Lewandowsky, 1899; Boruttau, 1899; Loewi, 1908), Sawyer and Schlossberg (1933) have recently found that if the instillation is carried on for a sufficiently long time it produces changes in the size of the pupil which differ according to the strength of the dose and the period of instillation. Smaller doses and shorter instillations cause a slight contraction of the pupil, while larger doses and longer instillations cause a contraction followed by dilatation.

In the present investigation, in most cases the standard doses of adrenalin which were chosen produced in the normal iris only a contraction of the constrictor muscles. Those most frequently employed were a 1/10,000 solution (in distilled water), instilled for 30 minutes, or a 1/2,000 solution, instilled for 5 to 10 minutes. The usual effect produced by this instillation was quite constant in all the normal animals, namely, a decrease in the horizontal measurement of the pupil of the instilled eye by 0.5 mm., while that of the control eye measured 2 mm. The measurement of the horizontal diameter of the pupil in all cases was made by holding a millimeter scale close in front of the eye, with the light so adjusted that the control pupil measured 2 mm. throughout the experiment.

The effect of pancreatectomy on the response of the iris to adrenalin. After the response of the normal iris to the standard dose of adrenalin had been established in each animal, the pancreas was removed and subsequent changes in pupillary size were noted. The reaction of the iris to adrenalin after the removal of the pancreas was studied in seventeen animals (table 3). In all cases the results were consistent, i.e., a mydriasis occurred in the instilled eye after pancreatectomy. Usually on the first day after the operation there was no change. Often on the second day adrenalin caused a definite dilatation which was more marked if insulin had not been given. If insulin was withheld longer, the effect of adrenalin increased strikingly on the third and fourth days, when it reached a maximum. At this time it was necessary to readminister insulin to save the life of the animal. The maximal pupillary widths of the instilled eyes of sixteen diabetic animals are seen in table 3 and vary from 3.5 to 9 mm., the control eye in all cases reading 2 mm. This mydriasis usually does not appear for some minutes (15 to 30) after the instillation. In many cases the first effect noticed is a slight pupillary contraction similar to that seen in the normal animal. The mydriasis which follows reaches a maximum usually in about two hours and may last for three or four hours or, when very marked, for five or six hours. In most cases at the time of maximal dilatation insulin had been withheld for several days and acetone bodies were present in the urine. Readministration of insulin in doses which caused the acetone bodies to disappear in a day or two greatly decreased the adrenalin mydriasis. In most animals, however, insulin treatment only diminished the mydriasis

TABLE 3

Comparison of the effect of adrenalin on the width of the pupil under different conditions

CAT NUMBER	STRENGTH OF ADRENALIN	TIME OF INSTILLATION	EFFECT OF ADRENALIN BEFORE PANCREATECTOMY		MAXIMAL EFFECT OF ADRENALIN AFTER PANCREATECTOMY		DAYS OFF INSULIN TO OBTAIN MAXIMAL EFFECT	ACETONE BODIES AT TIME OF MAXIMAL READING	EFFECT OF ADRENALIN AFTER INSULIN	REMARKS
			Control pupil	Instilled pupil	Control pupil	Instilled pupil				
		minutes	*mm.*	*mm.*	*mm.*	*mm.*			*mm.*	
S6	1:10,000	20	2.0	1.5	2.0	7.0	4	++		Eyes normal
S32	1:10,000	35	2.0	1.5	2.0	6.0	4	+++	Mydriasis 1	Eyes normal
S13	1:10,000	30	2.0	1.5	2.0	5.0	3	+		Eyes normal
S12	1:10,000	30	2.0	1.5	2.0	7.0	3	+++	Mydriasis 5, decreased to 2	Nictitating membrane removed
S10	1:10,000	30	2.0	1.5	2.0	6.0	3	+	Mydriasis 4, decreased to 2	Nictitating membrane removed
S16	1:10,000	3	2.0	3.5	2.0	6.5	3	+++	Mydriasis 2.5 to contraction 0.5	Sympathetic nerve cut in neck, nictitating membrane removed
S21	1:10,000	30	2.0	1.5	2.0	5.0	3	+		Eyes normal, died pneumonia
S22	1:10,000	30	2.0	1.5	2.0	3.5	2	++	Mydriasis 0.5	Eyes normal, died hypoglycemia
S50	1:2,000	10	2.0	1.5	2.0	7.0	19 hrs.		Mydriasis 0.5	Eyes normal, very fat cat died on 2nd day, extremely dehydrated
S12	1:10,000	30	2.0	1.5	2.0	9.0	2	++		Adrenals inactivated
S10	1:10,000	30	2.0	1.5	2.0	8.0	2	++++	Mydriasis 3	Adrenals inactivated

TABLE 3—*Concluded*

CAT NUMBER	STRENGTH OF ADRENALIN	TIME OF INSTILLATION	EFFECT OF ADRENALIN BEFORE PANCREATECTOMY		MAXIMAL EFFECT OF ADRENALIN AFTER PANCREATECTOMY		DAYS OFF INSULIN TO OBTAIN MAXIMAL EFFECT	ACETONE BODIES AT TIME OF MAXIMAL READING	EFFECT OF ADRENALIN AFTER INSULIN	REMARKS
			Control pupil	Instilled pupil	Control pupil	Instilled pupil				
		minutes	*mm.*	*mm.*	*mm.*	*mm.*			*mm.*	
S20	1:10,000	7	2.0	1.5	2.0	4.5	3	++++	Mydriasis 1	Adrenals inactivated before pancreatectomy
S38	1:2,000	10	2.0	1.5	2.0	7.0	2	+	Contraction 0.5	Thyroidectomized before pancreatectomy
S39	1:2,000	5	2.0	1.5	2.0	4.5	6	+++		Portal vein tied, thin animal
S42	1:2,000	10	2.0	1.5	2.0	5.5	6			Portal vein tied, thin animal
S45	1:2,000	7	2.0	1.0	2.0	2.5	4			
S90	1:2,000	10	2.0	1.5	2.0	3.5	6	+++++	Contraction 0.5	Portal vein tied

and did not completely abolish it or bring back the normal response obtained before pancreatectomy. A typical experiment showing a graphic representation of pupillary changes before and after removal of the pancreas is seen in table 4. In this table the drawings are made exactly to scale from the horizontal and vertical diameters of the pupils as obtained by direct measurement.

The effect of partial removal of the pancreas (about nine-tenths) was studied in several animals. Although they rapidly lost weight and had glycosuria they did not show acidosis and survived for some weeks without insulin. The adrenalin mydriasis did not occur in these animals.

ATTEMPTED ANALYSIS OF THE ADRENALIN MYDRIASIS SEEN AFTER PANCREATECTOMY. 1. *The effect of adrenal inactivation and denervation of the liver.* The effect of adrenal inactivation (removal of one gland and denervation of the other) and denervation of the liver was studied in three

TABLE 4

Comparison of the effect of adrenalin on the width of the pupil before and after pancreatectomy

Cat S12. Adrenalin (1:10,000) instilled into left eye for 30 minutes.

DATE	PUPIL		INSULIN	ACETONE BODIES IN URINE	SUGAR IN URINE
	Control	Instilled	*units per day*		*grams per day*
November 5					
Pancreas removed November 9					
November 11			8*	+++	9.3
November 12			8	+	
November 13			8	++	
November 14–18			6–12		1.1–1.5
November 18			14		1.5
November 20			12	+	3.1
November 20–23			14	+	1.6–3.6
Adrenals inactivated November 23					
November 24				+	0.5
November 25			5	+++	2.3

* After reading taken on eye.

animals. In two of these the inactivation was performed after and in the third before removal of the pancreas (table 3). In cat S10 the maximal difference in the pupillary diameter between the control and instilled eye after removal of the pancreas was 4 mm. Administration of insulin de-

creased this difference to 2 mm., but after subsequent adrenal inactivation and withdrawal of insulin for two days the difference increased to 6 mm. Similarly, in S12 (table 4) the maximal mydriasis obtained after pancreatectomy, insulin having been withheld for three days, was 5 mm. This difference decreased to 2 mm. with insulin treatment, but after adrenal inactivation and no insulin for two days it increased to 7 mm. Consistent results were obtained in S20, in which a difference of 2.5 mm. was observed after inactivation of the adrenals and removal of the pancreas. These three experiments justify the conclusion that the adrenals are not responsible for the sensitization of the iris to instilled adrenalin after pancreatectomy.

2. *The influence of the thyroid.* After the adrenals had been eliminated as factors in sensitizing the iris to adrenalin, it seemed important to ascertain whether increased thyroid activity was responsible for the phenomenon, especially as Goetsch (1918) had furnished evidence for an increased sensitivity to adrenalin in hyperthyroidism, and Loewi (1908) and others had observed adrenalin mydriasis in a few such cases.

Two types of experiments were tried: 1, the effect of thyroidectomy before pancreatectomy; and 2, the effect in normal animals of thyroxin injection and thyroid feeding on the response of the iris. In table 5 is given the protocol of an experiment in which the thyroid was removed completely before studying the eye reactions. At the first mydriasis was not so marked as usual, but after several periods of administering and withholding insulin, it became quite definite.

In two normal animals the influence of thyroxin injections was studied. In one cat 10 mgm. of thyroxin administered 3 to 4 hours before the instillation of adrenalin had no effect. In a second animal 2.6 and 3.2 mgm. given on two successive days likewise had no effect. Negative results were obtained also in a cat to which 3.88 mgm. of thyroid extract had been fed daily for a period of ten days.

From the above experiments it did not seem likely that hyperactivity of the thyroid was responsible for sensitization of the iris to instilled adrenalin.

3. *The influence of the liver.* It has been demonstrated that an adrenin-like substance is released from the liver on stimulation of its sympathetic supply (Cannon and Uridil, 1921; Cannon and Griffith, 1922; Rosenblueth and Cannon, 1932). In consideration of the possibility of increased liver activity in diabetes, a few experiments were done in which the portal vein was tied previous to the pancreatectomy, in an attempt to lessen or rule out the influence of this gland. The portal ligation was done in two stages: in the first operation the vein was about two-thirds tied off, and in the second operation after two weeks the tie was completed and the pancreas removed. These animals were able to survive several days longer without insulin than any in the previous experiments, but they eventually showed an acidosis.

TABLE 5

Comparison of the effect of adrenalin on a thyroidectomized animal before and after pancreatectomy

Cat S38. Adrenalin (1:2,000) instilled into left eye for 10 minutes. Thyroid removed completely March 19.

DATE	PUPIL		INSULIN	ACETONE BODIES IN URINE	SUGAR IN URINE
	Control	Instilled			
			units per day		*grams per day*
March 29					
March 30					
Pancreas removed April 8					
April 9			6	Slight +	8.8
April 11			None		1.8
April 12			None	++	8.8
April 13			20*	+++++	
April 14			None	+++	0.7
April 15			None	+++++	
April 16			20**	+++++	3.4
April 17			None		0.6
April 18			14*		0.5

* After reading taken on eye.

** Before reading.

The adrenalin mydriasis in these animals was, on the whole, not so marked as in the other groups (see table 4). The maximal differences in

width of the pupils in the control and instilled eyes in five animals was between 1.5 and 3.5 mm. The appearance of the maximal mydriasis usually coincided with the appearance of the acidosis as in most of the other experiments. The delayed onset of acidosis in these animals is probably due to their being thinner than normal animals at the time of pancreatectomy, because of the previous operation.

Discussion. The data reported above lead to the conclusion that the hypersensitivity of the iris and n.m. to adrenalin in experimental diabetes does not result from increased activity of the sympathico-adrenal system, since it occurs after inactivation of the adrenals. This conclusion is in accord with the work of La Barre and Houssa (1932), who found that the amount of adrenin is decreased in the circulating blood of diabetic animals off insulin. Nor is the hypersensitivity due to secretion of sympathin from the liver or thyroxin from the thyroid gland.

An alternative explanation of this phenomenon is that it results from general changes occurring in diabetes which affect the permeability of the conjunctiva or the rate of absorption of instilled adrenalin. For example, Straub (1910) showed that inflammation of the eye structures and subsequent vaso-dilatation increase the effect of both myopic and mydriatic drugs. The hyperlgycemia of diabetes has been offered as the explanation of the Loewi reaction by Loewi and Rosenberg (1914), because they obtained an increased sensitivity of the iris of normal animals after administration of a hypertonic glucose solution. This explanation, however, seems to be not valid because in the present investigation partially depancreatized animals which showed a continuous glycosuria and hyperglycemia did not exhibit a positive Loewi reaction, provided acidosis was absent. Apparently a close relationship exists between the development of an acidosis and the sensitization of the eye structures to adrenalin, although the two cannot be definitely correlated. For example, all the maximal eye responses occur at the time of the most severe acidosis or a day or so later. Furthermore, in very fat animals in which an acidosis sets in rapidly a marked sensitization may appear within 19 hours after pancreatectomy (table 3, S50), whereas normally it does not occur for at least 24 to 36 hours. On the other hand, it does not appear that the acidosis *per se* is the cause of the Loewi phenomenon, because a moderate reaction is observed frequently after several days' administration of insulin when the symptoms of acidosis are entirely lacking.

It is known that a condition of dehydration accompanies diabetic acidosis. Atchley, Loeb, Richards, Benedict and Driscoll (1933) observed in diabetic patients a loss of intracellular and extracellular body water and salts on withdrawal of insulin. This process started before symptoms of acidosis set in and increased as the acidosis increased. Other investigators also have stressed the importance of dehydration and hemo-concentration in

diabetic acidosis (Peters, Bulger and Eisenman, 1925; Peters, Kydd and Eisenman, 1933). In the present investigation it was observed that the animals in acidosis which showed a maximal Loewi reaction and sensitization of the n.m. appeared extremely dehydrated. This condition was especially apparent in the tissues surrounding the eyeball. It is possible that loss of water and salts from these tissues changes their permeability so that more adrenalin is absorbed and hence its effect of the iris and n.m. is increased. On this basis, the occurrence of a positive Loewi reaction in normal animals after injection of large amounts of hypertonic glucose solution could be explained (Loewi and Rosenberg, 1914) as well as its occurrence in other pathological conditions than diabetes mellitus (Lepehne and Schlossberg, 1924).

SUMMARY

A sensitization of both the nictitating membrane and iris to adrenalin occurs invariably in cats after complete removal of the pancreas, provided insulin is withheld (tables 2 and 3).

This reaction is absent after partial removal of the pancreas when, without insulin, hyperglycemia and glycosuria are present but acidosis is absent (see p. 498).

The sensitization of the iris to adrenalin in very fat animals takes place more rapidly than in those which are normal, whereas in thin animals the effect is delayed.

The sensitization of the iris and nictitating membrane is not influenced by inactivation of the adrenal glands or denervation of the liver (tables 2, 3 and 4).

Complete removal of the thyroid gland as well as ligation of the portal vein do not abolish the sensitization of the iris (tables 2 and 5).

The conclusion is drawn that the Loewi reaction and the sensitization of the nictitating membrane are not to be explained by increased discharge of adrenin in diabetes; it is suggested that they result from secondary factors accompanying diabetic acidosis that affect the permeability of the eye tissues to adrenalin.

This problem was suggested by Prof. W. B. Cannon, to whom I am indebted for much helpful advice.

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